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Methicillin-resistant *Staphylococcus aureus* in Southwest Finland

Changing strain types and antimicrobial resistance – methods for outbreak investigation

Jaakko Silvola

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
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Teoreettikkoin vaatimukset ovat siis tässä kohdin
sekä mahdottomia että – osaksi tarpeettomiakin.

J.J. Karvonen, 1900, Duodecim

UNIVERSITY OF TURKU
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ABSTRACT

Staphylococcus aureus is a gram-positive, coccus-shaped bacterial species. It is a major opportunistic pathogen of humans and typically associated with purulent skin and soft tissue infections and invasive infections. It is asymptomatically carried by some third of the population in the anterior nares, skin or mucosa. Methicillin-resistant *S. aureus* (MRSA) is a strain with acquired broad-spectrum resistance to most β-lactams, key antibiotics in the treatment of staphylococcal infection. Treatment of MRSA infections is hence challenging. Combined with an effective asymptomatic transmission in healthcare institutions and the community, MRSA inflicts a long-lasting and significant burden on healthcare systems and human health worldwide.

The aim of this thesis was to characterize transmission and risk factors of MRSA isolates exhibiting *spa* t172, reported as the most prevalent strain type during a period of increasing notification rate in Southwest Finland, 2007–2016 (Study I). We also aimed to analyse the changes in the antimicrobial susceptibility pattern of all MRSA isolates detected during the same period (Study II). Lastly, we aimed to evaluate the performance of a novel rapid bacterial strain typing tool, Fourier Transform Infrared Spectroscopy (FTIR) for outbreak detection and typing of *S. aureus* isolates, 2023–2024 (Study III).

Using whole-genome sequencing, we identified multiple clusters among the *spa* t172 isolates. Clusters of t172 were detected from healthcare and community sources in epidemiologically unlinked individuals, with identification dates spanning multiple years (Study I). During 2007–2016, the increase of MRSA notification rate was accompanied by an increasing trend of multi-resistant strains, driven by increases in resistance to erythromycin, clindamycin and tetracycline - and associated with community-acquisition, immigration and hospital care abroad (Study II). With the FTIR method we were able to detect multiple phenotypic subtypes of *S. aureus* sharing the same *spa* type, while the MRSA and MSSA isolates overall exhibited a high degree of phenotypic similarity (Study III). In conclusion, the assessment and adaptation of new laboratory-based analyses together with epidemiological investigation are crucial to limit the health impact of MRSA. Research of the MRSA transmission dynamics and changing pattern of antimicrobial resistance can provide information for targeted, local infection control measures and updated treatment guidelines.

KEYWORDS: MRSA, strain typing, whole genome sequencing, Southwest Finland

TURUN YLIOPISTO
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Staphylococcus aureus on grampositiivinen kokkibakteeri. Se on yleinen opportunistinen taudinaiheuttaja, joka liittyy tyypillisesti märkäisiin iho- ja pehmytkudos-infektioihin sekä syviin infektioihin. Noin kolmasosa väestöstä kantaa bakteeria oireettomasti sierainten etuosassa, iholla tai limakalvoilla. Metisilliiniresistentti *S. aureus* (MRSA) on bakteerin antibiooteille resistentti muoto. MRSA on hankkinut laajakirjoisen vastustuskyvyn stafylokokki-infektioiden hoidossa käytetyille β-laktaamiantibiooteille. MRSA-infektioiden hoito on vaativaa. MRSA leviää terveydenhuollossa ja yhteiskunnassa tehokkaasti aiheuttaen maailmanlaajuisesti merkittävän tautitaakan.

Tämän väitöskirjan tavoitteina oli tutkia Varsinais-Suomessa 2007–2016 yleisen MRSA kantatyypin (*spa* t172) tartuntareittejä ja kantaan liittyviä riskitekijöitä. Tutkimus keskittyi vuosiin, jolloin MRSA:n ilmaantuvuus oli alueella nousussa (Osatyö I). Lisäksi selvitimme MRSA-kantojen mikrobilääkeresistenssin muutosta samalla ajanjaksolla (Osatyö II). Viimeisessä osatyössä tavoitteena oli arvioida uuden, nopean bakteerityypitysmenetelmän: Fourier-muunnos infrapunaspektroskopian (FTIR), suorituskykyä *S. aureuksen* tyypityksessä ja epidemiantunnistuksessa, 2023–2024 (Osatyö III).

Bakteerin kokogenomin sekvensoinnilla tunnistimme useita t172-kantojen tartuntaketjuja. Samankaltaisia kantoja havaittiin toisiinsa linkittymättömillä potilailla, jopa vuosien aikaerolla (Osatyö I). Ilmaantuvuuden noustessa tutkimusjaksolla havaittiin myös moniresistenttien MRSA-kantojen osuudessa nousua. Tämä näkyi erityisesti erytromysiini-, klindamysiini- ja tetrasykliiniresistenssin kasvuna. Moniresistenssi liittyi avohoitoperäisiin kantoihin ja ulkomaan kontakteihin (Osatyö II). FTIR-menetelmällä osoitimme useita, ilmiasultaan erilaisia alatyyppejä *S. aureus* -kannoilla, joilla oli sama *spa* tyyppi. Herkät *S. aureus* -kannat ja MRSA-kannat puolestaan olivat yleensä hyvin samankaltaisia (Osatyö III). Lopuksi; uusien laboratoriomenetelmien arviointi ja käyttöönotto yhdessä epidemiologisen analyysin kanssa ovat avainasemassa, kun MRSA:n leviämistä halutaan ymmärtää ja torjunta suunnata oikein. MRSA:n leviämistapojen ja mikrobiolääkeresistenssin muutoksen tutkimus on tärkeää hoitosuositusten suunnittelussa.

AVAINSANAT: MRSA, kantatyypitys, kokogenomin sekvensointi, Varsinais-Suomi

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Abbreviations

AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
BORSA	Borderline oxacillin resistant *Staphylococcus aureus*
BSI	Bloodstream infection
BURP	Based-upon repeat pattern
CA-MRSA	Community-associated MRSA
CC	Clonal complex
CLSI	Clinical & Laboratory Standards Institute
CoNS	Coagulase-negative staphylococci
CP	Capsular polysaccharide
CWA	Cell wall anchored
cgMLST	Core-genome multi-locus sequence typing
DALY	Disability-adjusted life year
EARS-Net	European Antimicrobial Resistance Surveillance Network
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FC	Family cluster
FiRe	Finnish Study Group for Antimicrobial Resistance
FTIR	Fourier-transform infrared spectroscopy
HA-MRSA	Healthcare-associated MRSA
HAI	Healthcare-associated infection
HAO	Healthcare-associated outbreak
HD	Hospital district
IQR	Interquartile range
IWG-SCC	International Working Group on the Staphylococcal Cassette Chromosome elements
LA-MRSA	Livestock-associated MRSA
LDA	Linear discriminant analysis
LTCF	Long-term care facility
MALDI-TOF	Matrix-assisted laser desorption/ionization-time of flight
MDR	Multi-drug resistant
MH	Müller-Hinton (agar)

MIC	Minimum inhibitory concentration
MLST	Multilocus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Mass spectrometry
MSSA	Methicillin-susceptible *Staphylococcus aureus*
NCBI	National Center for Biotechnology Information
NIDR	National infectious diseases register
NT	Nontypeable
PCA	Principal component analysis
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
PRSA	Penicillin-resistant *Staphylococcus aureus*
PSSA	Penicillin-susceptible *Staphylococcus aureus*
PVL	Panton-Valentine leucocidin
SAB	*Staphylococcus aureus* bacteraemia
SCC*mec*	Staphylococcal cassette chromosome *mec*
SNV/P	Single nucleotide variant/polymorphism
SO	Suspected outbreak
SSTI	Skin and soft tissue infection
ST	Sequence type
THL	Terveyden ja hyvinvoinnin laitos, Finnish Institute for Health and Welfare
TSA	Tryptic soy agar
VISA	Vancomycin-intermediate *Staphylococcus aureus*
VRSA	Vancomycin-resistant *Staphylococcus aureus*
WGS	Whole-genome sequencing
WHO	World Health Organization
WSC	Wellbeing service county

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Silvola J., Gröndahl-Yli-Hannuksela K., Hirvioja T., Rantakokko-Jalava K., Rintala E., Auranen K., Junnila J., Marttila H., Lindholm L. & Vuopio J. (2022) Whole genome sequencing reveals new links between *spa* t172/CC59 methicillin-resistant *Staphylococcus aureus* cases in low-endemicity region of Southwest Finland, 2007–2016. *Scientific Reports*, 12:21326 https://doi.org/10.1038/s41598-022-25556-w

II Silvola J., Gröndahl-Yli-Hannuksela K., Hirvioja T., Rantakokko-Jalava K., Kanerva M., Auranen K., Marttila H., Junnila J. & Vuopio J. (2024) Increasing trend of antimicrobial resistance among methicillin-resistant *Staphylococcus aureus* strains in Southwest Finland, 2007-2016: An analysis of shifting strain dynamics and emerging risk factors. *Journal of Global Antimicrobial Resistance*, 40:47–52 https://doi.org/10.1016/j.jgar.2024.11.015

III Silvola J.*, Harju I.*, Kallonen T., Gröndahl-Yli-Hannuksela K., Kanerva M., Vuopio J., Rantakokko-Jalava K. (2026) Evaluating the performance of Fourier transform infrared spectroscopy for typing and outbreak investigation of methicillin-resistant and -susceptible *Staphylococcus aureus. Microbiology Spectrum,* 0:e03245-25. https://doi.org/10.1128/spectrum.03245-25

*Equal contribution

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1 Introduction

Staphylococcus aureus is a gram-positive coccus, an opportunistic pathogen and a frequent commensal coloniser of the skin and mucosa of both humans and animals[1]. *S. aureus* can cause a range of infections, from mild skin and soft tissue infections (SSTIs) and toxin-mediated disease such as food poisoning, to endocarditis, spondylodiscitis, osteomyelitis and sepsis[2]. In invasive infection, *S. aureus* can disseminate hematogenously and form metastatic abscesses in multiple body sites. The most common infections are SSTIs, whereas invasive infections and bacteraemia are often associated with medical procedures and foreign bodies, such as central and peripheral venous catheters[3]. *S. aureus* is one of the leading causes of healthcare-associated infections and surgical site infections[3]. In 2020, *S. aureus* was the second most common cause of bloodstream infections in Finland, and approximately half of these were healthcare-associated infections (HAIs)[4]. Methicillin resistant *S. aureus* (MRSA) strains exhibit broad-spectrum antimicrobial resistance (AMR) to β-lactams, complicating the treatment of infections[5]. MRSA inflicts a notable disease burden worldwide[6,7].

MRSA is a notifiable finding in Finland, which has remained a low-endemicity region. However, since 2020 an increase in the overall MRSA notification rate and bloodstream infections (BSI) has been observed every year until 2024 (21.6 to 29.5 and 1.2 to 1.8/100,000 people, respectively) with 3% of invasive *S. aureus* isolates now MRSA[8]. The same is true for Southwest Finland, where a significant increase of MRSA notification rate was initially reported between 2007–2016 (12.4 to 24.9/100,000 people), surpassing the national average by 2024 (34.6/100,000 people)[8,9]. While the risk factors of MRSA acquisition differ between countries, observational studies in Finland have identified immigration, hospital care abroad, MRSA exposure within household, occupational exposure to livestock-associated MRSA (LA-MRSA) and injection drug-use as possible risk factors contributing to the increasing notification rate[9–12].

The objectives of this thesis were to understand the changing demographic and strain-related factors contributing to the changing epidemiology, outbreaks as well as antimicrobial resistance of MRSA in the low-endemicity setting of Southwest Finland. These objectives are explored by the application of both new and

conventional staphylococcal typing methods and epidemiological analysis in three studies (I-III). In Study I we analysed the diversity and transmission of endemic MRSA t172/CC59 isolates using whole-genome sequencing (WGS). In Study II we characterized the strain type and AMR profiles of circulating MRSA strains in Southwest Finland, 2007–2016 and identified risk factors of AMR. In Study III we evaluated the performance of FTIR as a rapid strain typing tool to complement WGS and *spa* typing in staphylococcal typing and outbreak investigation.

2 Review of the Literature

2.1 Characteristics of *Staphylococcus aureus*

This section briefly reviews the features of *S. aureus* as a pathogenic micro-organism including taxonomic definition, microbiological and clinical characteristics, and susceptibility to antibiotics.

2.1.1 Taxonomic definition

Staphylococcus aureus is a bacterial species of the *Staphylococcaceae* family[13]. The etymology of the name are the Greek word *stafylókokkos* (gr. σταφυλόκοκκος, ‘’bunch of grapes’’) and Latin word *aurum* (‘’golden’’) which refer to the aniline-violet-stained groups of cocci seen under the microscope and the yellow pigment of the culture colonies, respectively (**Figure 1**). These names are historically credited to the work of Ogston, who in 1881 named the clustered micrococci *Staphylococci* and described its role in sepsis and abscess formation, and Rosenbach, who in 1884 named an isolated, pigmented bacterial colony *Staphylococcus pyogenes aureus*[14,15].

Staphylococcus and *Micrococcus* genera were originally placed in the *Micrococcaceae* family based on phenotypic characteristics, together with other gram-positive, catalase positive cocci. Later, in phylogenetic analysis it was revealed that staphylococci are not very closely related to *Micrococcaceae* family (*Micrococcales* order), but part of an entirely different order: *Caryophanales*, class: *Bacilli*, phylum: *Bacillota* (synonym: *Firmicutes*)[16,17]. Low content of G+C (30 to 39%) in the genome is characteristic for staphylococci. The *Staphylococcus* genus now includes 71 validly published and correctly named species (as of December 2025[18]).

Nowadays, phylogenomic studies have demarcated conserved signature indels to separate *S. aureus* from other species of the *Staphylococcaceae* family and *Gemella* species[19]. Additionally, recent studies on staphylococci have revealed novel closely related (87% to 94% average nucleotide identity) bacterial species, such as *S. argenteus, S. roterodami, S. schweitzeri* and *S. singaporensis*, which are currently not properly differentiated in the clinical microbiology laboratory by routine identification methods such as matrix-assisted laser desorption/ionization time-of-

flight mass spectrometry (MALDI-TOF MS) or conventional biochemical profiling (see chapter 2.3.2)[20,21]. Confusion can be mitigated with the adaptation of the species complex concept *i.e. Staphylococcus aureus* complex – which refers to these closely related species that share phenotypic characteristics. The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) currently discourages distinguishing between the aforementioned species of *S. aureus* complex in clinical reporting to avoid confusion, especially with the coagulase negative staphylococci (CoNS), and recommends the use of *S. aureus* complex instead[22]. In the following chapters, the term *S. aureus* is used in reference to the species included in the *S. aureus* complex, unless otherwise specified.

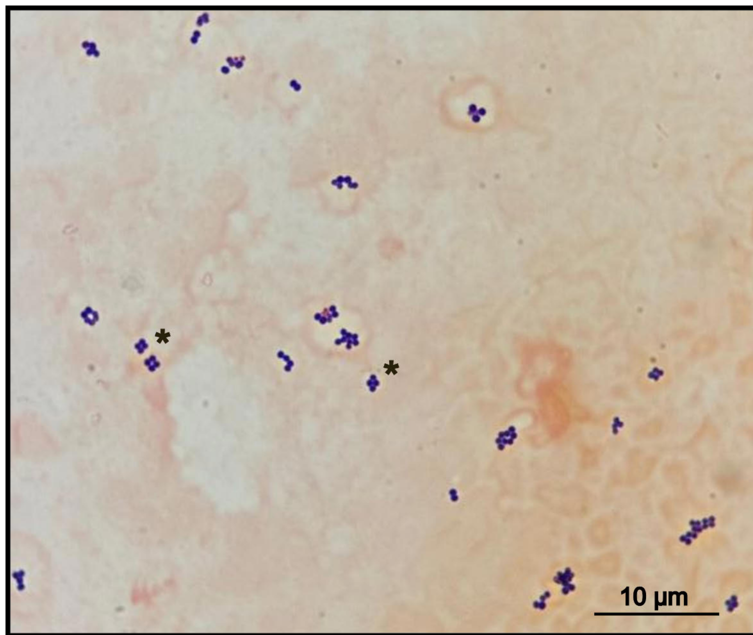

Figure 1. Gram-staining showing *S. aureus* in a clinical anaerobic blood culture, 100 x magnification, approximated scale. Typical irregular groups of gram-positive cocci as well as a chain, pair and tetrad formation (asterisks) can be observed. Author's photo.

2.1.2 Structure and cell wall

Under the microscope, *S. aureus* appears as spherical, non-spore-forming and typically gram-positive coccus with cell size of approximately 0.5 to 1.5 µm in diameter[2]. The bacterium is facultatively anaerobic, meaning growth can be observed in the presence or absence of oxygen, and not capnophilic, meaning it does not require high concentration of carbon dioxide to thrive[23]. It characteristically forms groups of several up to hundreds of individual cocci adhered together in

groups. In liquid culture media, formation of pairs, short chains and tetrads resulting from dividing bacterial cells can be observed (**Figure 1**)[2]. The bacterium is nonmotile and tolerant to broad pH range and salinity, making it well adapted to asymptomatically colonise the skin and mucosa of humans and animals (as a commensal) as well as environmental surfaces and objects[24]. *S. aureus* can also shift from being a harmless commensal to a pathogen and cause endogenous infections when the host immunity is compromised[25].

The cell wall of *S. aureus* is composed mostly of highly cross-linked peptidoglycan and ribitol-/lipoteichoic acid[2]. It is a dynamic and complex structure accommodating requirements for the upkeep and modification of the cellular shape, as well as host and environment interactions[26]. The cell-wall peptidoglycan of *S. aureus* has specific, defining structural characteristics that contrast other gram-positive and -negative organisms: short average glycan chain length, thickness, high-degree of peptidoglycan cross-linking (80–90% of the available stem peptides) and high rigidity[27].

The cell wall of *S. aureus* is primarily synthesized and remodelled by a species-specific family of enzymes known as the penicillin binding proteins (PBPs), the target of β-lactam antimicrobials[28]. The main functions of PBPs are to catalyse the polymerization of the glycan strands and cross-linking of forming glycan chains, the core structures of the staphylococcal cell wall[29]. Individual β-lactams have differences in their spectrum and (therapeutic) level of activity where they are able to inhibit the function of PBPs[30]. Importantly, the altered PBP2a transpeptidase facilitates the synthesis of and upkeep of the *S. aureus* cell wall when other PBPs are incapacitated by β-lactam antimicrobials[31]. The acquisition of PBP2a via its encoding gene, *mecA* or *mecC*, hence is the basis for the MRSA phenotype of *S. aureus* (see chapter 2.1.6)[28,32].

Outside the cell wall, *S. aureus* exhibits a polysaccharide capsule (CP), a virulence factor of most clinical strains, promoting a variable degree of antiphagocytosis[33]. CP is the target for serotyping (see chapter 2.3.8.1).

2.1.3 Virulence factors

S. aureus has adapted to survive in different environments and expresses a variety of known virulence factors (*e.g.* toxins, cell wall components, adhesins, enzymes, immunomodulators) promoting within-host survival and contributing to its ability in causing clinical infections[2,34]. While the determinants of virulence in *S. aureus* are diverse, the expression of most virulence factors is regulated by the signalling-pathway system encoded in the *agr* locus, and its variants[35,36].

S. aureus is catalase positive[2]. Catalase, a hydroperoxidase enzyme, has long been implicated as an important diagnostic marker and a virulence determinant of *S.*

aureus, as it decreases the bactericidal activity of polymorphonuclear neutrophils via deactivation of secreted hydrogen peroxide[37]. Another characteristic of the bacterium used in standard identification is the production of plasma coagulase (coagulase tests)[2]. *S. aureus* secretes two soluble proteins that interact with prothrombin to catalyse conversion of plasma fibrinogen to fibrin[38]. Coagulase test is of clinical importance, as it can be used to distinguish *S. aureus* from the commonly occurring and less virulent CoNS, although careful interpretation of the test result is warranted as some other species of staphylococci (*e.g. S. pseudintermedius*) are known to also exhibit a positive test result[39].

S. aureus exhibits a plethora of covalently linked cell wall anchored (CWA) proteins that are vital in its ability to cause clinical carriage and infection[2]. One well established CWA protein is the surface protein A encoded by the *spa* gene, which contributes to the survival of the bacterium within tissues by promoting tissue invasion and inhibition of phagocytosis by binding the tail (Fc) part of secreted immunoglobulins, especially IgG, impairing their function[40,41]. The *spa* gene is widely used in staphylococcal typing (see chapter 2.3.5).

The microbial surface components recognizing adhesive matrix molecules are an abundant family of CWA proteins known to contribute to the virulence of *S. aureus*[42]. Most importantly, they facilitate the adherence and biofilm formation of the bacterium in tissues (collagen- and fibrinogen binding) which in turn forms the basis for the pathogenesis of staphylococcal abscess formation[42,43].

Secreted toxins of *S. aureus* drive the pathogenesis of toxin-mediated staphylococcal disease[2]. The expression of hemolysins (*hlb, hla, hlgC, hld*) is characteristic for most clinical *S. aureus* strains (β-haemolysis observed on blood agar, **Figure 2**) and a combination of the effect of multiple hemolysins is required for complete reaction to occur on blood agar[44]. Staphylococcal food poisoning is associated with the expression of enterotoxins (*e.g. sea*), working as superantigens[45]. The exfoliative toxins of *S. aureus* are serine proteases recognizing and hydrolysing the desmosome cadherins in the epidermis of humans[46,47]. Expression of exfoliative toxins has been associated with specific human disease affecting the skin, such as bullous impetigo, staphylococcal scalded-skin syndrome (*eta, etb*) affecting infants and the toxic shock syndrome (*tsst-1*)[36].

One important secreted, pore-forming toxin, is the Panton-Valentine leucocidin (PVL)[48–50]. While PVL is characteristic for many community associated MRSA lineages (CA-MRSA, see chapter 2.2.4), expression of the toxin has been observed in multiple lineages of *S. aureus*, including methicillin-susceptible *S. aureus* (MSSA) strains[51]. For example, in an Australian study, 98% of CA-MRSA strains carried genes encoding PVL[48]. The structure of PVL is similar to that of Hla, and the main target of PVL is polymorphonuclear leukocytes (neutrophils), while subsequent release of pro-inflammatory substances promote also thrombosis[49]. The

toxin is composed of two subunits with specific determinants, *lukF-PV* and *lukS-PV,* which are spread by both clonal expansion and horizontal transfer (via bacteriophages)[52]. Although experimental data on the role of PVL in staphylococcal pathogenesis is partly controversial, PVL-positive MRSA strains have repeatedly been associated with serious clinical manifestations, such as necrotizing skin and soft tissue infection, pneumonia and sepsis[34,53]. Abscess formation in SSTI and invasive infection are common clinical issues linked with PVL-positive MRSA and MSSA strains[50,54].

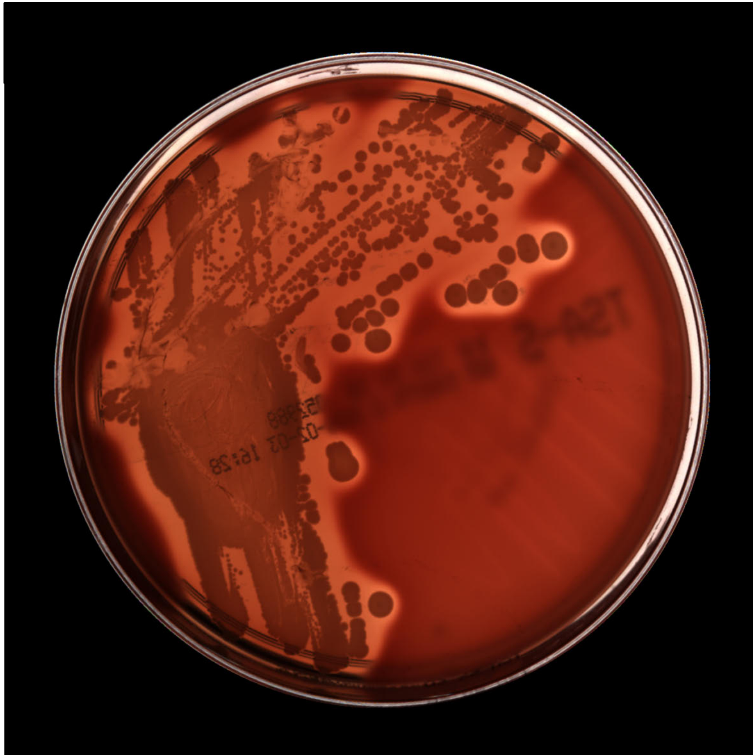

Figure 2. *S. aureus* grown on blood agar. Complete β-haemolysis present as a light halo around the colonies. Author's photo

2.1.4 Genome

The genome of *S. aureus* ranges in size from 2.8 to 2.9 Mbp, with a G+C content of approximately 32.7%[55,56]. In a comparative genomic analysis of 13 human-associated *S. aureus* genomes, the supragenome (*i.e.* pangenome) contained 3,221 genes, of which 2,245 were (shared) core genes, whereas *Haemophilus influenzae* and *Streptococcus pneumoniae* pangenomes were larger (5,170 and 3,783 genes

respectively), but shared fewer core genes than *S. aureus*[57]. The authors concluded that *S. aureus* has as smaller pool of horizontally acquired genes, but a larger core gene pool, possibly indicative of a longer co-evolution with the human host and hence a relatively smaller selection pressure, compared to the other included species inhabiting the similar naso-/oropharyngeal niche. Nonetheless, *S. aureus* genomes usually include multiple mobile DNA elements, including both conjugative and nonconjugative plasmids, transposons, prophages and pathogenicity islands[58,59].

Driven by the increasingly economically feasible adaptation of WGS for epidemiological surveillance and research, the number of published *S. aureus* genomes has increased dramatically during the last few years[60]. Currently there are 119,618 *S. aureus* draft genomes in the Genome database available at National Center for Biotechnology Information (NCBI)[61]. Excluding atypical and metagenome-assembled genomes, the number is 119,367 (as of December 2025)[62]. In the European counterpart, European Nucleotide archive, there were 115,542 assembled genomes tagged with the *S. aureus* taxid 1280 (as of December 2025)[63].

2.1.5 Clinical characteristics

2.1.5.1 Asymptomatic carriage

S. aureus is a frequent asymptomatic colonizer, a commensal, of humans and animals[25]. While almost everyone will be exposed to *S. aureus* during their lifetime, the estimates on persistent and intermittent carriage rates vary[64,65]. The preferred carriage site in people is the nasal cavity, where at any given moment up to 20–30% of people are colonized[25,66]. Carriage in the oropharynx has been suggested as an alternative primary niche, with a similar stability of carriage as the nose[67]. In animals, the emergence of livestock-associated MRSA (LA-MRSA) ST398 has sparked interest in studies, where colonisation of humans, livestock (especially pigs, but also dairy cattle, chicken, horses, turkeys) and pets with similar strains have been described[68].

Nasal colonization with *S. aureus* is a dynamic process not fully understood. It is influenced by the interplay between host immune factors and competition with other microbes occupying the same niche[25,69]. While up to 20% of people appear to be colonized persistently, others are able to clear repeated introduction of *S. aureus* in the nose, which is reflected in the distinct antistaphylococcal antibody profiles between persistent and occasional carriers[70]. The staphylococcal protein A has been identified as a possible driver of nasal carriage, as it has been observed among the most abundant immunoevasive exoproteins secreted by carrier strains[41].

In addition to the nasal cavity, *S. aureus* can be carried elsewhere on the skin, such as axillae, throat, the groin and perineal area and the gastrointestinal tract[71].

Intestinal carriage of *S. aureus* can be observed in the absence of nasal colonisation, but appears to be rarer than combined (nasal and intestinal) carriage[72]. While the bacterial load and number of colonised body sites are associated with increased risk of staphylococcal infection, sole extranasal colonisation was not found an independent risk factor for *S. aureus* postoperative infection in a recent large cohort study looking at 3,369 carriers, suggesting the importance of the nasal site in the pathogenesis of endogenous *S. aureus* infection[73].

Persistent nasal carriers are also known to be at risk of invasive infection, especially in the immunocompromised or hospitalised patient population[64,74]. Elimination of *S. aureus* carriage has been suggested as a strategy to reduce the likelihood of infection, since the colonizing strains often are able to cause clinical infections[64]. This has been observed especially in the surgical patient population: preoperative elimination of *S. aureus* carriage reduced postoperative staphylococcal infection among patients receiving prosthetic joints[75]. More broadly, identification of nasal and extranasal carriers upon admission, and subsequent eradication treatment for patient expected to be hospitalized for a prolonged period (weeks), has been be used as an effective strategy to reduce hospital-acquired, surgical site *S. aureus* infections[76].

2.1.5.2 Infections and treatment

S. aureus harbours a unique array of adaptive and virulence determinants which contributes to its ability in causing variable clinical manifestations[44]. *S. aureus* infections range in severity from typically mild, superficial skin and soft tissue infections to severe and life-threatening septic infections, and toxin-mediated disease[2,46]. *S. aureus* is a notorious and common cause of invasive infection, where the tendency and ability to spread haematologically from the primary infection focus to different body sites and cause abscesses is pathognomonic[2,34,77]. Management of *S. aureus* infection is often complicated by adherence to medical devices (*e.g.* central and peripheral venous catheters, prosthetic joints) and persistent bacteremia[77].

SSTIs are the most common infection type associated with *S. aureus*, and often caused by strains circulating in the community (*e.g.* households[50]). SSTIs caused by *S. aureus* are usually purulent (*i.e.* discharging pus) and manifest commonly as abscesses (including furuncles and carbuncles), cellulitis and impetigo[54]. More severe manifestations include the formation of deep abscesses, osteoarticular infections (osteomyelitis, spondylodiscitis) and infective endocarditis[78]. The presence of *S. aureus* bacteriuria has been identified as an independent risk factor of endocarditis and admission to ICU in those without urinary tract origin of the *S. aureus* bacteraemia (SAB), proposed as a possible marker for deep tissue dissemination and thus warranting further diagnostics[79].

Recently, multiple *S. aureus* strains have shown epidemic transmission among injection drug users in the USA, where injection drug use has been increasing[80,81]. Infections in this demographic tend to show significantly increased risk of invasive MRSA (over MSSA aetiology), endocarditis, septic embolism, osteomyelitis, recurrent invasive infection and increased mortality[10,82]. In Finland, a similar phenomenon has been observed and recently associated with multiple MRSA strains, *spa* types t008 and t024, while the most prevalent *spa* type among this demographic in Southwest Finland was t386[10]. These observations reflect the high transmissibility of *S. aureus* strains among injection drug users and possibly suggest that the current public health measures are insufficient to prevent these severe infections[83].

S. aureus can colonize and associate with biofilms (together with CoNS and other bacteria) on implanted medical materials and devices, making patients with prosthetic valves, joint prosthesis, central and peripheral venous catheters at risk of a foreign-body associated infection, especially following *S. aureus* bacteraemia[77,84,85]. *S. aureus* is also a frequently isolated pathogen from surgical site infections (other than colon surgery), and a major cause of all healthcare-associated infections[3,86]. In the EU/EEA, 2018–2020, *S. aureus* was identified in 15.2% of all surgical site infections reported to ECDC (n=11,170), surpassed only by *Enterococcus* spp. (17.6% proportion) and *E. coli* (17.2% proportion) which were more frequently associated with abdominal surgery[3]. In Finland, 2024, *S. aureus* was identified in 46.8% of surgical site infections with an identified microbiological cause (n=427); and in 23.1% of all blood culture positive, healthcare-associated infections (n=2,041)[86]. In addition to infections originating from central and peripheral venous catheters and surgical site infection, hospital-acquired- or ventilator-associated pneumonia caused by *S. aureus* contributes to the healthcare-associated infection burden[87]. Although *S. aureus* has been recognized as a possible cause of community-associated pneumonia, its proportion there is far surpassed by pneumococcus[88].

Bacteraemic infection with *S. aureus* is associated with significant mortality: a recent meta-analysis observed an estimated mortality of 10.4% at 7 days, 13.3% at 1 month, 27.0% at 3 months and of 30.2% at 1 year[89]. Methicillin-resistance increases the mortality attributable to SAB[89,90]. Unlike many other conditions inflicting a high overall mortality and morbidity, little improvement has been occurring in the outcome of SAB during the last decades, even though some new therapeutic alternatives, such as dalbavancin, daptomycin, ceftaroline and ceftobiprole, have been introduced[91]. Infectious diseases consultation, however, has been shown to reduce 5-year composite outcome (all-cause mortality or recurrence of SAB; adjusted hazard ratio 0.71; 95%CI 0.68–0.74, $p<0.001$), reflecting the complexity in the successful management of severe *S. aureus* infection[92]. Invasive infection is usually managed with intravenous β-lactams (staphylococcal penicillins) or

glycopeptides (vancomycin), if MRSA is suspected, or local prevalence rates are high[93].

Source control is an important part of SAB management[2,94]. Persistent bacteraemia, defined as >2 days or more despite active antimicrobial therapy, is associated with significantly increased mortality (adjusted hazard ratio 1.93, 95%CI 1.51–2.46, $p<0.001$) and warrants active screening of infectious foci, possible surgical intervention and removal of infected medical devices such as venous catheters[95]. Recently, Prosty *et al.* conducted a review and meta-analysis on cefazolin and staphylococcal penicillins for the first-line treatment of SAB[96]. They observed non-inferiority of cefazolin, which was associated with reduced odds of 30-day all-cause mortality (odds ratio 0.73, 95%CI: 0.62–0.85). Analysis of discontinuation due to adverse events or toxicity favoured cefazolin. However, there was no randomized data published, and the authors assessed a moderate-high risk of bias in the included 30 observational studies. Additionally to the uncertainty regarding the currently available optimal antimicrobial regimen for the management of SAB, there is lack of sound evidence regarding the optimal treatment length[97].

Recently, the reported re-emergence of penicillin susceptible *S. aureus* (PSSA) strains have sparked discussion about the revival of penicillin in the treatment of SAB, as some data has suggested a more favourable outcome of severe infection in comparison with the staphylococcal penicillins[98]. In the Pirkanmaa area (since 2023: The Wellbeing services county of Pirkanmaa), Finland, the proportion of PSSA among invasive *S. aureus* infections was increasing between 2007–2015, reaching 43.1% in 2015[99]. More recent data from Sweden showed, that up to 35.1% of SAB could be now caused by PSSA, and unlike to the population structure of MRSA, the PSSA strains seem to represent a diverse collection of clonal lineages[100]. Penicillin susceptibility is currently not routinely determined for clinical *S. aureus* strains everywhere in Finland.

The treatment of superficial or mild *S. aureus* infections in the community usually consists of the use of a peroral β-lactam/β-lactamase-inhibitor combination, staphylococcal penicillin, or even topical agents such as fusidic acid and mupirocin[93,101]. If an abscess is present, surgical intervention with incision and drainage are generally recommended, as antibiotics penetrate poorly within the abscess[94].

2.1.6 Resistance to β-lactams

Beta-lactams are key antimicrobials in the treatment of staphylococcal infection[94]. They are a versatile group of antibiotics and provide effective bactericidal effect to susceptible *S. aureus* strains in low concentrations[102]. Penicillins tend to have a relatively short half-life and hence have to be administered multiple times per day to

achieve sufficient concentration and time above the bactericidal minimum inhibitory concentration, MIC (*e.g.* see the European Committee on Antimicrobial Susceptibility Testing, EUCAST rationale document for benzylpenicillin[103]).

The basis for the structure and function of all β-lactams is the β-lactam ring structure whereas variation in the core ring structure and side chain determinants effects the microbiological spectrum and β-lactamase tolerance of the agent[104,105]. Taking account the possible addition of β-lactamase inhibitors, these can be divided by their activity into narrow- and broad-spectrum antimicrobials[105]. While a multitude of different molecules are known, four major classes of clinically applicable β-lactams are usually referred to: penams/clavams (*i.e.* penicillins), cephems (*i.e.* cephalosporins and cephamycins), penems (*i*.e. carbapenems) and monobactams (*i.e*. aztreonam) - with a tendency of increased activity towards Gram-negative organisms towards the latter[106].

The function of β-lactams is based on the disruption of bacterial cell wall peptidoglycan synthesis, specifically by covalently binding, and inhibiting the action of cell wall transpeptidases, PBPs, resulting in loss of cell wall integrity and eventually to the lysis of the microbial cell[104]. Bacteria have evolved several mechanisms to resist this effect[30]. Resistance to β-lactams can be intrinsic or acquired, and primary resistance mechanisms used by Gram-negative (expression of β-lactamases) and Gram-positive (PBP target modifications) organisms are usually different, albeit not categorical[107].

S. aureus employs two primary strategies to avoid the function of β-lactams: 1) production of enzymes, *i.e.* β-lactamases, to break down the β-lactam ring rendering the drug ineffective and 2) target mutation, changing the three-dimensional structure of the PBPs, resulting in reduced binding affinity of β-lactams into their target[5,108]. Resistance to β-lactams in *S. aureus* is mediated by the horizontal acquisition (recombination events) of mobile genetic elements, such as plasmids and the staphylococcal cassette chromosome *mec* (SCC*mec*) containing resistance genes, or via adaptive mutations[109–111].

The most important staphylococcal β-lactamase, encoded by the *blaZ* gene often located in a plasmid, emerged shortly after the introduction of penicillin in the 1940s (see chapter 2.2.1)[108,112]. Penicillin resistance in methicillin-susceptible *S. aureus* (MSSA) isolates is mainly driven by the *blaZ*-mediated β-lactamase production[5,100]. These enzymes represent the molecular class A, functional subgroup 2a, of bacterial serine-β-lactamases and exhibit a relatively narrow-spectrum of hydrolytic activity towards penicillin and its derivatives (aminopenicillins); and are inhibited by the most common β-lactamase inhibitors clavulanic acid and tazobactam[113]. Five variants of the staphylococcal penicillinase are characterized, and one of them (variant F) has recently been shown to confer the borderline oxacillin resistant *S. aureus* (BORSA) phenotype[108]. The BORSA phenotype, characterized by oxacillin

MIC range of 1–8 mg/L, can risk treatment failure when using antistaphylococcal penicillins, and on the other hand sometimes leads to false identification of an MSSA strain as MRSA[114]. The prevalence of BORSA strains is variable from population to population, with an estimated average of 5%[114]. Nowadays, up to 70% of clinical *S. aureus* isolates are resistant to phenoxymethylpenicillin (Penicillin V) and aminopenicillins, although some studies have indicated that the penicillin-susceptibility might be increasing, as discussed above[115,116].

Another possible β-lactam resistance phenotype, sometimes called the modified *S. aureus,* is associated with changes in the native PBPs of *S. aureus* or expression of drug efflux transporters[117]. These strains are negative for *mecA/mecC* and can exhibit either an MRSA or MSSA phenotype in the routine AST - providing a challenge for laboratory detection[117,118].

The broad-spectrum β-lactam resistance in *S. aureus* – the MRSA phenotype – results from the presence of a modified cell-wall transpeptidase PBP2a, encoded by the *mecA* or *mecC* gene, located within the *mec* gene complex in the MGE called SCC*mec*[116,119,120]. The transpeptidase action of the acquired PBP2a in cell-wall synthesis is not inhibited in the presence of most β-lactams, unlike native PBPs of *S. aureus,* and together with the native PBPs, is sufficient to enable the survival of MRSA bacteria in the presence of β-lactams[31,121].

The *mecA* gene complex includes regulatory genes *mecI* and *mecR1,* and both the sequences and the organization of these are homologous with the *bla* gene complex[122]. The *mecA* gene complex is located within a 20–56 kb insertion sequence designated the staphylococcal cassette chromosome *mec* (SCC*mec* element), which is inserted within the *orfX* (an RNA methyltransferase) gene of *S. aureus*[1,32]. The SCC*mec* element was first characterized by Ito *et al.*[123], who cloned and determined the structure of a chromosomal region, absent in MSSA.

A new *mecA* gene homolog (68.7% identity to *mecA* in *S. aureus* strain N315), was first reported in 2011 from both humans and bovine MRSA isolates[124]. These isolates were negative in a polymerase chain reaction (PCR) -test for *mecA,* showed phenotypic methicillin-resistance and the most prevalent strain type was CC130-t843[124]. This *mecA* homolog became known as *mecC*[125]. A modified PCR test was developed to detect *mecC*[126]. The *mecC* has since been associated with the animal reservoir, especially ruminants: in Finland, a *mecC* prevalence of 1.5% was reported among bovine MRSA isolates in 2013[127]. A large study in Denmark identified only two *mecC*-positive MRSA strains before 2003, but a total of 110 isolates 2003–2011, associating *mecC* detection with CA-MRSA, older patient population than other MRSA cases, contact to livestock and rural areas[128].

The SCCmec element is mobile, which is mediated by site-specific integration/excision by recombinase genes ccrA and ccrB[123]. The evolutionary origin of the SCCmec element is not completely clear, but it is shared by the CoNS and S.

aureus; multiple staphylococcal species, namely S. sciuri, S. vitulinus and S. fleurettii, have played a role in the assembly of the mec gene complex and the element has probably been originally transferred via a recombination event from S. sciuri, where the SCCmec is ubiquitous[129]. The clonal nature of successful epidemic MRSA lineages suggests that the horizontal acquisition of the SCCmec element has occurred only a limited number of times, albeit horizontal transfer of the element between different S. aureus strains in people living in the same household has been described[1,110].

SCC*mec* element types are used to define specific MRSA clones, usually in combination with a genotyping result such as sequence type (ST) or *spa* type (*e.g.* MRSA-ST8-IV, see chapter 2.3.641)[130]. An international consensus was proposed and largely adapted in 2009 by the Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC), for reporting[122]. The SCC*mec* elements (marked with roman numerals) are classified in a hierarchical system, affected by two key structures within the SCC*mec*: the *ccr* gene complex type (*ccr* allotype) and the *mec* gene complex[122]. Currently, fifteen SCC*mec* types (I–XV) have been described, with the latest addition from food isolates, China[131]. SCC*mec* types I, II, IV and V currently have subtypes, and in 2021 the IWG-SCC decided not to further annotate new SCC*mec* subtypes in other species than *S. aureus,* due to increasing complexity[132].

2.1.7 Other mechanisms of antimicrobial resistance

While β-lactams are clinically the most important antistaphylococcal drugs, infections caused by resistant strains can be treated with a variety of alternative antibiotics depending on the clinical manifestation and phenotypic susceptibility profile[93,94]. The complete picture of resistance mechanisms in *S. aureus* is diverse, and resistance can occur with either an acquired or adaptive route[111]. In the outpatient setting for mild infections, peroral treatment options are needed[133]. While vancomycin has remained important in the treatment of invasive infections, some data suggest the adoption of newer, less nephrotoxic alternatives, such as daptomycin[134,135]. Some characterized AMR mechanisms are summarized in **Table 1**.

Table 1. Some antimicrobial resistance determinants and mechanisms encountered in *Staphylococcus aureus*

ANTIBIOTIC GROUP	AFFECTED ANTIBIOTICS	RESISTANCE DETERMINANT(S)	RESISTANCE MECHANISM	REFERENCE(S)
BETA-LACTAMS	Penicillins (inc. aminopenicillins)	*blaZ* (acquired)	Enzymatic breakdown (beta-lactamase)	108
	Staphylococcal penicillins Cephalosporins Carbapenems	*mecA, mecC* (acquired)	Target modification (affinity loss)	120
GLYCO-PEPTIDES	Vancomycin (VRSA)	*vanA, vanB* (acquired)	Target modification (affinity loss)	136
	Vancomycin-intermediate (VISA)	Chromosomal adaptation (adaptive)	Cell-wall thickening	137
LINCOSAMIDES, MACROLIDES	Clindamycin, erythromycin (and other macrolides)	Erm-type ribosomal methylases, groups *erm*(A), *erm*(C) (acquired)	Target modification (affinity loss)	134
LINCOSAMIDES	Clindamycin	*linA, InuA* (acquired)	Enzymatic breakdown (3-lincomycin, 4-clindamycin O-nucleotidyltransfe rase)	138
AMINOGLYCO-SIDES	Gentamycin, amikacin, tobramycin	*aacA-aphD, aadD, aph(3')-IIIa* (acquired)	Enzymatic deactivation (aminoglycoside modifying enzymes)	139
SULPHON-AMIDES +/- TRIMETHOPRIM	Sulfamethoxazole-trimethoprim, sulfadiazine-trimethoprim, trimethoprim	*dfrB, dfrG* (acquired and adaptive)	Target modification (affinity loss)	140,141
TETRA-CYCLINES	Tetracycline, doxycycline, minocycline	tet(O), *tet*(M), *tet*(K), *tet*(L) (acquired and adaptive)	Multimodal: active efflux, target modification	142
FLUORO-QUINOLONES	Ciprofloxacin, levofloxacin, moxifloxacin	*grlA, grlB, gyrA, gyrB* (adaptive)	Multimodal: active efflux, target modification	143–145

2.1.7.1 Resistance to glycopeptides

Glycopeptides, including antimicrobials such as vancomycin, teicoplanin and newer oritavancin, telavancin and dalbavancin, have remained as the first-line treatment option for severe MRSA infections since the 1980s[94,146]. Glycopeptide resistance in *S. aureus* is hence of especial clinical concern. Vancomycin was made commercially available in 1958 by US pharmaceutical company Eli Lilly under the brand name Vancocin (**Figure 3**) and despite the initial challenges associated with the clinical use of vancomycin (poor oral bioavailability, inferior antibacterial activity in comparison with the β-lactams and oto-/nephrotoxicity of the early formulations), *S. aureus* appeared not to develop resistance to the drug very efficiently after repeated exposure *in vitro* – which had been experienced with the β-lactams[147].

In 1997 the first clinical isolate with a reduced susceptibility to vancomycin was reported[148]. The strain was identified from a relapsing surgical wound infection of a 4-month-old infant, following 29 days of continuous vancomycin therapy. An MIC of 8 mg/L for vancomycin was reported using a broth microdilution method, and the strain subsequently classified as intermediately resistant *S. aureus* (VISA). The absence of *vanA/vanB* was later confirmed with a PCR test. Subsequent analyses have associated the appearance of VISA strains with hospitalization, relapsing infection and prolonged exposure to vancomycin[146]. Increased vancomycin MIC (>1.5 mg/L) has been associated with increased all-cause 30-day mortality (odds ratio 2.59;95%CI 1.49–4.51, p=0.001)[149].

The first vancomycin-resistant *S. aureus* strain (VRSA), with a MIC of >32 mg/L for vancomycin, was reported in the USA, 2002 in a patient suffering from repeated diabetic ulcers of the lower extremity[136]. The vancomycin resistance of the VRSA strain was mediated by a plasmid-based production of cell-wall modifying enzymes resulting in reduced affinity of the vancomycin binding in its cell wall target - originating possibly via horizontal acquisition of the *vanA* operon from the colonizing *vanA*-positive vancomycin-resistant *Enterococcus faecalis* strain[136]. The VRSA phenotype has remained rare, and the first isolate from Europe was not reported until 2013, from Portugal[150]. Other than for VRSA, EUCAST currently discourages the use of disk diffusion, but recommends the determination of MIC with broth microdilution for all patients undergoing vancomycin therapy[5].

2.2 Epidemiology of MRSA

This section reviews the emergence, transmission, epidemiology, and the burden of disease estimates due to MRSA.

2.2.1 Emergence of resistance to β-lactams

Throughout the 20th century, the emergence and spread of AMR in *S. aureus* has repeatedly followed the introduction of new therapeutics, raising significant clinical concerns regarding the future of effective treatment (**Figure 3**). Reports of penicillin resistant *S. aureus* (PRSA) started to emerge shortly after the initial clinical trials, commercial introduction and upscaling of production of penicillin during the first years of the 1940s[112,151]. By the end of 1950s, PRSA was universal in hospitals and towards the end of 1960s, in the community[152]. By the mid-1970s, an estimated 90% of hospital, and 70–85% of community-acquired *S. aureus* isolates were considered PRSA, resulting in alternative treatment regimens and reduction in the use of penicillin as an antistaphylococcal drug[152]. Nowadays, native penicillins are re-emerging in the treatment of *S. aureus* infections (see chapter 2.1.5.2).

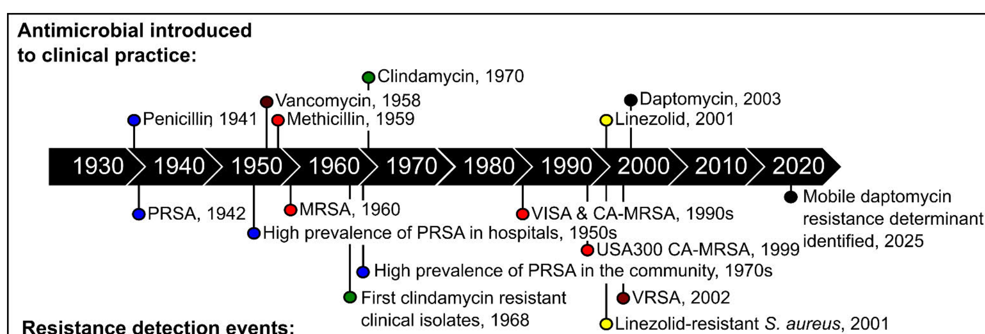

Figure 3. Timeline delineating introduction and resistance detection events of some antimicrobial agents with activity to *S. aureus*. PRSA Penicillin-resistant *S. aureus,* CA-MRSA Community-associated methicillin-resistant *S. aureus,* VISA Vancomycin-intermediate *S. aureus,* VRSA Vancomycin-resistant *S. aureus.* Summarized and adapted from multiple sources, mainly:[143,149]

Methicillin, a penicillin-derivative developed by the company Beecham in the late 1950s, was one of the first β-lactam antibiotics introduced to specifically surpass the effect of the staphylococcal penicillinase[153]. These compounds include an isoxazolyl side chain which renders them penicillinase-resistant, hence the name staphylococcal penicillins, or penicillinase-resistant penicillins[154]. In 1961, shortly after the introduction of methicillin to clinics, the first report on the detection of three methicillin-resistant isolates (MICs of 12.5–25 µg/ml) among 5,440 tested specimens of *S. aureus,* was published in the *British Medical Journal*[155]. Eventually successful MRSA strains spread globally: first detections remained in close association with healthcare institutions, but especially from the 1990s onward, widespread transmission in the community was apparent[156,157]. Staphylococcal

penicillins used nowadays include mostly oxacillin and other isoxazolyl-derivatives, but the nomenclature of MRSA has persisted from the original observations to date[93].

2.2.2 Transmission

MRSA is primarily transmitted through contact transmission (**Figure 4**). In the healthcare environment, transmission occurs primarily from patient to patient via hand carriage of healthcare personnel in direct contact, but also by contaminated objects (*i.e.* fomites such as medical equipment, clothes) and the environment[158]. Airborne transmission via excreted infectious respiratory particles is possible - and a seasonal pattern of MRSA infections is suggested, with a peak in both paediatric and adult infections seen in the spring and summer[159,160]. Healthcare personnel can become colonized and serve as reservoirs for MRSA in healthcare institutions, while evidence also suggest their role as possible sources for outbreaks[161,162]. Additionally, the risk of laboratory-acquired colonization and subsequent infection with an epidemic MRSA strain has been recognized[163].

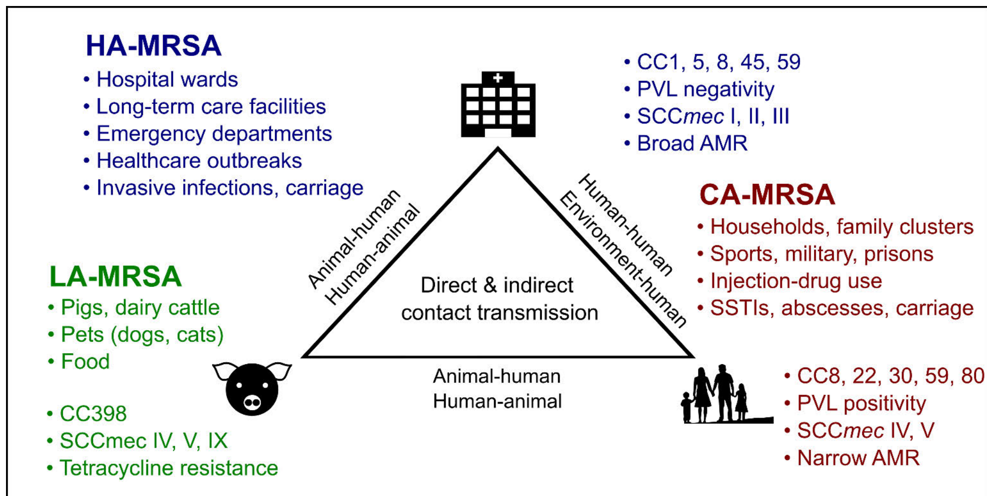

Figure 4. A schematic representation on the transmission of MRSA between different reservoirs, prevalent lineages (clonal complex, CC) associated with the epidemiological groups, associated staphylococcal cassette chromosome *mec* (SCC*mec*) types, characteristic presence of Panton-Valentine leucocidin (PVL), the spectrum of antimicrobial resistance (AMR) and clinical manifestations. Circulation of MRSA strains occur via a complex interplay of the environment (inc. fomites), humans and animals, primarily by contact transmission. HA-MRSA Healthcare-associated MRSA, CA-MRSA community-associated MRSA, LA-MRSA livestock-associated MRSA, SSTI skin and soft tissue infection. Summarized from multiple sources:[65,163,164]

MRSA can also effectively spread and persist in the community, where its acquisition has been associated with frequent physical contact and crowded living conditions (*e.g.* military camps[164]) as well as social and physical determinants of health, such as underserved populations, injection drug users[10], and people with chronic conditions (*e.g.* HIV and cystic fibrosis[1,165,166]). Frequent contact with livestock appears to be a risk factor for LA-MRSA acquisition, but invasive disease with these strains is not as common as with other MRSA strains[167]. Companion animals have also been recognized as a possible source of transmission and a reservoir within households[168].

2.2.3 MRSA: A healthcare-associated pathogen

MRSA is considered an important healthcare-associated pathogen, causing healthcare-associated infections (HAI) in a comorbid, high-risk patient population[86,169]. As defined by the ECDC, HAIs are characterized by symptom-onset on day 3 after admission to a given healthcare facility. In their latest point-prevalence study, ECDC estimated some 28,124 (95%CI 12,466–64,546) HAIs attributable to MRSA in the EU/EEA annually, 2022–2023[170]. In this study, Finland reported *S. aureus* as the most prevalent bacterial cause of HAIs with an identified microbiological cause, and the categorical MRSA proportion was <10 % (lowest category). Currently, the surveillance of AMR within the EU level is acknowledged and outlined by the EU legislation, where AMR is listed as a special health issue to be covered by epidemiological surveillance and relevant case definitions (Commission Implementing Decision 2018/945, 22.6.2018). This task is carried out by the European Antimicrobial Resistance Surveillance Network (EARS-Net) network within ECDC, where the clinical antimicrobial susceptibility data from national AMR surveillance initiatives is collected and analysed[171]. Promising recent trends have been described in the latest annual EARS-Net epidemiological report for 2024, where the estimated EU incidence of MRSA bloodstream infections was 4.48/100,000, a significant decreasing trend (20.4% decrease) from the baseline year 2019[171].

As opposed to MRSA appearing in the community (CA-MRSA), healthcare-associated strains (HA-MRSA) have traditionally been considered as separate epidemiological entities (**Figure 4**). These definitions, although nowadays blurring, can be useful to understand the background of MRSA epidemiology[1]. HA-MRSA strains, detected among HAIs or asymptomatic carriers, are conventionally characterized by shared traits including the carriage of relatively large SCC*mec* types I, II and III, which often attribute AMR to many non-β-lactam agent classes - whereas CA-MRSA strains have been associated with the smaller SCC*mec* elements IV and V and non-β-lactam susceptibility[172].

As stated previously, MRSA was first encountered in the United Kingdom in 1961 and is hence considered the oldest multi-drug resistant (MDR) pathogen with a worldwide occurrence[155,157]. Although the occurrence of sporadic, naturally acquired resistance was noted already in the 1960s in countries where penicillinase-resistant penicillins were not yet available (*e.g.* Poland[173]), the epidemic potential of MRSA was not understood until some years later[153,157].

The global epidemiology of MRSA has been described in the literature as waves, each wave characterized by the emergence and spread of a limited number of successful epidemic, healthcare-associated clones (HA-MRSA, first and second wave), eventually expanding outside the healthcare environment (third wave, CA-MRSA) and the recognition of the animal reservoir (fourth wave, LA-MRSA)[157]. The first wave, occurring in the 1960s and 1970s, primarily affected the United Kingdom, Denmark and some other European countries[174]. A limited number of MRSA detections were reported from a few hospitals in the USA and Australia, while no reports of detections yet appeared elsewhere in the world[175,176]. The first wave was largely caused by a HA-MRSA strain known as the Archaic clone exhibiting ST250-SCC*mec* I (*spa* types t008, t051)[176,177]. The Archaic clone isolates from United Kingdom and Denmark exhibited a high-degree of similarity with the later Iberian clone ST247-SCC*mec* I strain - and lacked the regulatory gene (repressor) *mecI*[174,177].

The second wave started in the 1980s, marked by the disappearance of the Archaic clone and emergence of a few highly successful HA-MRSA lineages, including previously undetected lineages and the descendants of Archaic clone (*e.g.* Iberian and Rome clones)[178]. During the second wave, HA-MRSA outbreaks within and between hospitals were described increasingly in the USA, Europe, Australia and Asia[157]. In the United Kingdom, an epidemic strain (EMRSA-1, similar to an early Australian epidemic strain) emerged in 1981, causing a series of large outbreaks affecting multiple hospitals and hundreds of patients in different surgical and medical units despite attempted prudent infection control efforts[179]. In Athens, Greece, the proportion of MRSA among hospital-acquired *S. aureus* isolates was rising quickly, from 32% to 50%, between 1986 and 1987[180]. In some hospitals, HA-MRSA became endemic, and similar clones or their descendants (such as ST5) are still isolated today, meaning the second wave of MRSA is, in that sense, still ongoing[181,182].

The SENTRY Antimicrobial Surveillance Program, established in 1997, has analysed global trends in SAB and proportion of MRSA in SAB[183]. In 1998, the rate of MRSA among SAB in the USA was estimated to be around 30%, while the respective proportion in a multi-centre study was 22.9 % for central and southern Europe, 61.3% for Hong Kong and 71.6% for Japan[184]. The authors observed that in many areas the proportion of broadly resistant (MRSA + resistance to four or more

antibiotic classes) strains was over 30%. Using pulsed-field gel electrophoresis (PFGE) and ribotyping, they were also able to confirm intercontinental spread of broadly resistant MRSA clones[184].

In contrast to the situation forming in southern Europe, the Nordic countries and the Netherlands were able to curb the rising MRSA notification rates, possibly by implementing stringent infection control policies with a target to eliminate MRSA from healthcare institutions[185,186]. These measures included most importantly screening of high-risk patients and implementing contact precautions -and during the following years, a decreasing prevalence was observed: in Denmark, the proportion of MRSA among hospital isolates remaining under 1% throughout the 1980s[187], in the Netherlands, an MRSA prevalence of 0.54% was observed between 1990–1992[188].

The number of MRSA isolates in Finland remained between 100–150 annually, corresponding to a notification rate of approximately 2–3.1 cases per 100,000 population, throughout the 1980s with a slight increase in detections in the beginning of 1990s[189,190]. Invasive infections remained rare, and active screening schema combined with strain typing enabled the characterization of the first epidemic strains affecting multiple healthcare facilities in the country[189]. These were descendants of internationally established clones, such as the Iberian HA-MRSA clone ST247 and its single-locus variant which affected the Turku University hospital and district institutions in subsequent outbreaks, 1991–1992[185,189]. These were the first described large MRSA outbreaks in the country, and at the time their control by implementing strict infection control measures was considered significant, as it was not completely clear whether a complete eradication of MRSA from regional healthcare institutions was possible[185].

By the end of 1990s and early 2000s MRSA rates had increased in the USA, and accounted for 30 to 50% of all nosocomial *S. aureus* isolates, although variation was significant between hospitals[191]. In Europe, between 2004–2006 in a study surveying hospital isolates from multiple institutions, MRSA rates were 28.3% in France, 20% in Germany, 36.4% in Italy and 21.6% in the UK[192].

Towards the 21st century, Finland also started to observe a significant increase in the notification rate of MRSA (from 2.3 to 11.5 /100,000 people annually between 1997 and 2002, and up to 27.9/100,000 people by 2004) and the proportion of MRSA among invasive isolates increased from <1% to 2.8% concurrently[193,194]. These trends were attributed to increasing MRSA incidence mostly in southern Finland, driven by outbreaks in long-term care facilities (LTCF) and later also acute care hospitals and marked by the emergence of multiple epidemic strains (probably originating from the spread of CA-MRSA), named according to their characteristic PFGE profile: *e.g.* FIN-16 (ST125-IA), FIN-4 (ST375-IV), FIN-7 (ST8-IV) and FIN-10 (ST45-IV/V)[193].

2.2.4 MRSA in the community

For most part of the 20th century, MRSA was considered as primarily nosocomial pathogen, with a limited number of clonal strains causing infections in healthcare-associated settings[195]. The surge of MRSA infections in the community was observed in the 1990s, when infections were repeatedly detected in previously healthy individuals (adults and children), including mostly SSTIs with or without abscesses, in contrast to HA-MRSA strains which had been associated with surgical site infections, pneumonia and bacteraemic infections affecting the elderly and more comorbid patient population[196]. Another worrisome manifestation, associated with the PVL-positive CA-MRSA, was necrotizing pneumonia seen in young immunocompetent adults[197]. Although reports of CA-MRSA were present in the US in the 1970s and 1980s (*e.g.* bacteraemic MRSA strains circulating among injection drug users, exhibiting different phage types and antibiogram to nosocomial isolates) the term was generally accepted and popularized in the 1990s[198]. The emergence of CA-MRSA marked the third wave of MRSA and like the second wave, is still ongoing[157].

CA-MRSA strains were initially defined as isolates obtained from non-carrier inpatients within 48 to 72 hours of hospitalization and were characterized by shared phenotypic and molecular characteristics: PVL positivity, small SCC*mec* types IV and V, attributing fewer resistance determinants for non-β-lactam agents than larger SCC*mec* elements, and harbouring the arginine catabolic element[199]. The emergence of CA-MRSA inflicted a significant increase in the notification rate and disease burden attributable to MRSA in the 21st century in most parts of the world, including the Nordic countries[200–202].

New risk factors for MRSA acquisition were identified: CA-MRSA was associated with crowded living conditions (military camps[164], prison inmates[203]), frequent physical contact (sports teams[166]) and underserved ethnic minorities (Australian aboriginals[54], Native American populations[204]). A CA-MRSA clone, defined by its PFGE pattern as USA300 (MRSA-ST8/t008-IVa) became rapidly prevalent in different parts of the USA in the first years of the 2000s[156]. For example, between 2000 and 2002, proportion of CA-MRSA increased among clinical isolates in a children's hospital in Memphis, Tennessee, from 38% to 63%, mostly driven by the emergence of USA300 clone[205]. In a 2011 US nationwide study (43 medical centres across the country with 2,093 MRSA isolates included), USA300 (*spa* t008) was the most common clone across all regions (1,269 isolates; 61% of all), causing 37% of all MRSA BSIs and often exhibiting narrow-spectrum drug resistance to non-β-lactams (3%)[206]. USA300 was observed in both rural and metropolitan areas, and one example of transatlantic transmission via healthcare personnel to Europe was documented[207].

In France, Germany and Switzerland, ST80/t044-associated lineage was among the most prevalent initially detected CA-MRSA clones[208,209]. Denmark experienced a rapid increase in the annual number of new MRSA cases in the beginning of the 2000s, with a large proportion of the new cases originating in the community due to the spread of MRSA-ST80[210]. In 2005 following the emergence of the European clone (MRSA-ST80-IV) in Denmark, USA300 lineage had also become established in the country[210].

In Finland, Salmenlinna *et al.* first reported on the rate of CA-MRSA on the national level, identifying 108/526 (21%) of new MRSA cases as CA-MRSA during 1997–1999[211]. As the notification rate of MRSA started to increase in Finland in the early 2000s, Kanerva *et al.* investigated the Finnish MRSA cases without an identified link to healthcare, 2004–2006, and found 298 (7.4%) cases matching CA-MRSA case-definition[201]. Among these, FIN-4 (MRSA-ST375/t172-IV) and FIN-11 (''European clone'' MRSA-ST80/t044-IV) lineages were the most abundant, with 56 (19%) and 42 (14%) cases respectively, followed by FIN-12 (MRSA-ST22/t022-IV; 6%) and the USA300 associated FIN-25 (MRSA-ST8/t008-IV; 6%) lineages[201]. All of the aforementioned clones were also identified among HA-MRSA cases, reflecting how the epidemiological definitions were blurring already in the 2000s.

The FIN-4 (MRSA-ST375/t172-IV) lineage was one of the first and most abundantly identified CA-MRSA clones in Finland (Mikkeli I and II clones, detected first in 1993 in Eastern Finland and subsequently across the country)[211,212]. Following its emergence, the *spa* type t172 MRSA has consistently remained among the most frequent CA-MRSA clones detected in Finland since the routine adaptation of *spa* typing in 2009; up until very recently in the late 2010s, when its proportion has been slowly decreasing[8,213]. In the other Nordic countries, t172 was concurrently rarely detected[202]. The clone is characterized by susceptibility to non-β-lactam antimicrobials, PVL negativity and the carriage of SCC*mec* type IV, exhibiting significant epidemic potential[201]. The Finnish ST375/t172 clone is related to the successful CA-MRSA CC59 lineage (ST375 is a single locus variant of ST59) which has a worldwide occurrence[200]. CC59 has been divided into two major subclades: North America and East-Aasia although the strain is much more prevalent in East-Asia than in the USA[214]. Representatives of both branches are detected in Europe: and the CC59/t437 subclone has likely been imported from East-Asia[214,215]. In China, CC59 (mainly ST59/t437-IV) represented up to 30–40% of all MRSA isolates collected from multiple tertiary hospitals continuously during 2014–2020 and the ST59/t172 subclone was increasingly reported in some provinces[216].

2.2.5 Recent MRSA epidemiology

During the last decades, classical CA-MRSA strains have continued to increase among the hospitalized patient population, further blurring the line between conventional community/hospital epidemiological classification[217,218]. Within healthcare, CA-MRSA strains appear to efficiently acquire broader AMR profiles[200]. On the other hand, as more complex infections have increasingly been treated at home, the HA-MRSA lineages have ''escaped'' the healthcare setting, and are now circulating among the general population[200].

Finland and other Nordic countries have been able to keep MRSA infections somewhat rare in the healthcare setting despite the increasing notification rate following the emergence of CA-MRSA[3,219]. This has been thought to represent an outcome of an active surveillance and infection control policy present in the countries[200,220]. However, a worrisome trend during the 2010s has been observed across all the Nordics: the annual notification rate of MRSA has again been on the rise throughout the 2010s and 2020s, with a momentarily decrease during the COVID-19 pandemic (**Figure 5**)[202].

Some drivers of the increasing MRSA notification rate in the Nordics include immigration from higher-incidence areas (*spa* types t304 and t223 associated with asylum seekers from the Middle-East in Sweden[221], Finland[222] and Norway[223]), widespread transmission of LA-MRSA (CC398) in pigs and in the community in Denmark[224] and healthcare outbreaks. In Finland, for example, a prolonged, large healthcare-associated outbreak with the *spa* t067 strain affected multiple healthcare institutions and up to 2,778 patients (79% of all MRSA cases in the area during 2001–2014) in Pirkanmaa, southern Finland[225]. As described in chapter 2.1.5.2, MRSA cases have recently also increased among the injection drug users in Southern Finland[10]. Similar findings have been reported from Denmark, where two distinct MRSA outbreaks caused by t5147/ST88 and t1476/ST8 strains among injection drug users in Copenhagen, 2014–2020, were reported[226].

Recently in 2025, a fusidic-acid resistant, ST121, *spa* t272 CA-MRSA clone with an international occurrence was described, associated with impetigo and other SSTIs of young children[227]. The authors state, that the strain was detected primarily in Europe with largest number of isolates reported from the Netherlands, Denmark, France and Belgium while one isolate was reported from Finland, obtained from a British tourist. Interestingly, there was marked seasonality, with a clear peak in reporting during late summer and early autumn observed on multiple years[227]. The ST121 and related strains have been associated with impetigo outbreaks previously, likely related to the presence of mobile *eta, etb* and *edinC*, encoding exfoliative toxin A and B and epidermal cell differentiation inhibitor C, respectively[228]. More recently, evidence of intercontinental transmission and the capability of the strain to cause invasive disease, has been published[229].

Currently, armed conflicts pose a significant risk on the development and global spread of AMR, including MRSA[230]. Effects of the ongoing conflict in Eastern Europe between Russia and Ukraine on the AMR incidence, via *e.g.* patient transfers, has been observed in many countries including Finland[231].

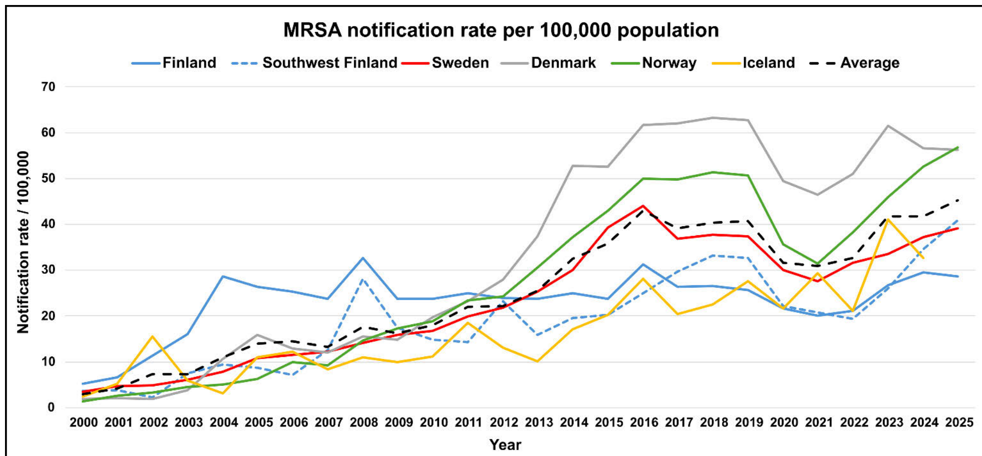

Figure 5. MRSA notification rate per 100,000 population in the Nordics, 2000–2025. Compiled and adapted from the infectious diseases registry data publicly available as annual reports and/or online from Finnish Institute for Health and Welfare (THL)[7], Folkhälsomyndigheten[228], Statens Serum Institut[229], Folkehelseinstituttet[230] and Directorate of Health, Iceland[231].

2.2.6 Burden of disease

In 2022, the most comprehensive systematic analysis outlining the global burden of disease due to AMR until then, including MRSA, was published[232]. Using various data sources and modelling (contrafactual of resistant infections replaced by susceptible), the authors estimated that approximately 1,270,000 deaths were directly attributable to AMR in 2019. Although significant variation in the availability and quality of collected data between regions was observed, the estimated number of deaths directly attributable to drug-resistant *S. aureus* in 2019 (n=178,000, including MRSA and strains with resistance to non-β-lactam antimicrobial agents) was exceeded only by that of drug-resistant *Escherichia coli* (n=219,000) and *Klebsiella pneumoniae* (n=193,000) globally. In an updated 2024 review by the same initiative, the authors estimated some 1,140,000 global deaths directly attributable to AMR in 2021[233]. They analysed AMR trends from 1990 to 2021 and found that among 22 pathogens and 84 pathogen-drug combinations, MRSA showed the largest increase in both associated and attributable deaths, reaching 550,000 associated and 130,000 attributable deaths in 2021.

Looking at the direct mortality inflicted by AMR does not reflect the complete scope of the issue. In addition to direct mortality, significant morbidity and economic sequelae are inflicted by AMR. Cassini *et al.* estimated, that in the EU/EEA, infections caused by MDR pathogens attributed 874,541 disability-adjusted life years (DALYs, 170/100,000 population) in 2015 alone, which is similar to the burden inflicted by influenza, HIV and tuberculosis combined (183/100,000 population annually, 2009–2013)[6,234]. Of the DALYs inflicted by MDR pathogens in the countries of EU/EEA in 2015, a median of 19.2% (32.6/100,000) were caused by MRSA, surpassed only by that of 3rd generation cephalosporin-resistant *E. coli* (37.2/100,000).

In Finland, the complete DALYs attributable to MDR pathogens were 47.82/100,000 population, of which MRSA attributed <10/100,000 population in 2015[6]. The burden of invasive MRSA in Finland has remained limited: in 2010, the incidence of HAIs due to MDR pathogens was relatively low (estimated 0.53/100,000 population), and the proportion of MRSA among these was far surpassed by 3rd generation cephalosporin-resistant *Enterobacteriaceae*[219]. Among BSIs, SAB has been identified as a growing health issue in the aging population, but the proportion of MRSA has remained between 2–3% annually[235].

2.3 Laboratory diagnostics and staphylococcal typing

This section briefly reviews the diagnostics and typing strategies of *S. aureus*. Appropriate diagnostic methods for MRSA detection, accompanied by infection control guidelines and control measures taken, are considered important to maintain a low MRSA prevalence in healthcare[236].

The main aim of staphylococcal strain typing is to define relatedness between isolates[237]. Typing is usually required in the context of infection control, surveillance or research and has one of two primary aims: 1) to define similarity of individual isolates in an outbreak (outbreak vs non-outbreak isolates) or 2) to identify broader transmission, ancestry and evolution of epidemic clones[156,237]. Several methods, genotypic and phenotypic, for staphylococcal strain typing have been developed throughout the years, with differences in performance, reproducibility, throughput and cost dictating the choice of the methods[60,238]. As in the cases of matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF) and antimicrobial susceptibility testing (AST), the diagnostic methods can sometimes be used or adapted for staphylococcal typing[239,240].

2.3.1 Culturing

When grown on blood agar, *S. aureus* forms typical colonies ranging from white to yellow in colour (**Figure 2**). Usually, preliminary identification on a blood agar plate includes the observation of a light halo surrounding the colony known as β-haemolysis (the breakdown of red blood cells) surrounding the bacterial colony. The ability of *S. aureus* to lyse red and white blood cells was first described over a century ago[241,242]. In addition to rich media supplemented with sheep or horse blood, *S. aureus* can grow on several artificial nonselective culture media such as tryptic soy agar (TSA), Müller-Hinton agar (MH) or tryptone glucose yeast extract agar[243]. Nowadays, selective or chromogenic culture media offer rapid preliminary laboratory diagnosis of *S. aureus*[244]. The choice of culture media can be important for phenotypical typing methods (see chapter 2.3.8).

By supplementing the medium with antimicrobials, MRSA can be selected[245]. This is useful when the aim is to screen clinical and environmental specimens for multi-drug resistant organisms, and to increase detection sensitivity, incubation using an enrichment broth can be added[243]. However, the utility of enrichment broth in clinical diagnostics has recently been questioned: the use of enrichment broth for many tissue and fluid specimen rarely yield results affecting patient care, which in turn results in high number of tests needed to find true positives with this method[245]. One exception to this appear to be orthopaedic, prosthesis-related bone- and joint infections, where biofilms including CoNS and *S. aureus* play an important role[246].

2.3.2 MALDI-TOF

Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) has been adapted as one the most widely used methods for microbiological species identification in clinical microbiology laboratories[247,248]. In MALDI-TOF, the specimen is placed in an organic matrix to a sample plate, which is then dried and irradiated/heated with a laser, followed by sublimation and ionization of the sample-matrix, which in turn travels through a vacuum (time-of-flight tube) to a detector[247]. The measurements are visualised in a spectrum of mass to charge (*m*/z) ratios, which reflect the protein composition of a given specimen – corresponding to a species-specific fingerprint[247,249]. The adaptation of the method has marked a paradigm-shift away from slower and more complicated testing strategies involved in clinical microbiology, complementing and in many cases replacing historical methodology such as stain-based, culture-based, biochemical and antigenic profiling required for microbial identification[247].

MALDI-TOF has been proposed also for strain typing of *S. aureus*. A few studies have evaluated the ability of MALDI-TOF to identify *S. aureus* strains on the subspecies level: Wolters *et al.*[240] were able to determine characteristic *m/z* values

for 5 major HA-MRSA CCs (CC5, CC8, CC22, CC30 and CC45) and were able to type 60 MRSA isolates with high concordance to the multilocus sequence typing (MLST)-based typing. Josten *et al.*[250] analysed the spectra of 401 MRSA and MSSA strains and identified peptides corresponding to the CC-specific peaks in the MS spectra. They showed that peak shifts in the CC-specific spectra were detected with three different instruments, represented single nucleotide polymorphs (SNPs) in the genomes of different strains, and while the ribosomal proteins showed little variability, small, less characterized proteins (*e.g.* stress proteins) were the most variable group. Correct discrimination of isolates which are more closely related, has been more difficult: Lopes *et al.* [251] observed limited similarity (11%) between PFGE and MALDI-TOF-based typing in subset (12 isolates) of subclinical bovine mastitis *S. aureus* strains.

2.3.3 Molecular testing in diagnostics

Molecular diagnostic testing refers to the use of multiple different laboratory techniques to detect pathogen specific genetic material (DNA or RNA) in clinical samples. These approaches are based on either techniques based on the PCR to amplify pathogen specific target (also: nucleic acid amplification test) or next-generation sequencing techniques[252].

Advantages molecular methods offer include high sensitivity and specificity, relatively rapid turnaround time and ability for automation[253]. These tests have been shown to decrease the time to effective therapy and length of stay, and can thereby reduce mortality among bloodstream infection patients when combined with an effective antimicrobial stewardship policy[254]. One important limitation of the highly sensitive molecular methods is the assessment of clinical relevance, especially when detecting polymicrobial infections including pathogens with low virulence, such as CoNS or *Cutibacterium acnes*[255]. Additionally, detections of pathogens with molecular methods do not always indicate the presence of a viable organism[256]. For detection of MRSA (targets including the *mecA* gene and SCC*mec* insertion sequence *attB*), commercial molecular tests have shown high specificity and sensitivity[257]. The cost of molecular detection for MRSA could be reduced with pooling samples of an individual patient, warranted by careful validation for sufficient sensitivity[258]. Molecular detection of the *mecA* or *mecC* from a culture can be used to reliably detect methicillin resistance[126,259]. Direct detection of *mecA*/*mecC* from a clinical specimen has also been applied, with one study reporting 91.7% sensitivity for a direct real-time PCR test from a nasal swab when compared to a culture-based method[260].

Metagenomic next-generation sequencing (*i.e.* detection and sequencing of all [microbial] genetic material present in a given sample) can be used for pathogen

detection especially in the context of prior or ongoing antimicrobial use, and to overcome diagnostic challenges when other methods fail to provide a diagnosis[261]. Metagenomic testing for bacteria can reveal the infectious agent without prior knowledge or suspicion of the aetiology. One promising approach is to predict the antimicrobial susceptibility by recognizing AMR determinants in a single-organism specimen, as shown in the case of periprosthetic tissue collected in blood culture bottles[262].

2.3.4 Antimicrobial susceptibility testing

Determining the antimicrobial susceptibility (antimicrobial susceptibility testing, AST) of organisms detected in clinical specimens is a core task in clinical microbiology. The Finnish Study Group for Antimicrobial Resistance (FiRe), established in 1992 and coordinated by THL, aims to produce reliable and comparable information (*e.g.* annual epidemiological reports, FinRES reports) on the AMR of clinically important organisms[263]. FiRe includes 21 Finnish clinical microbiology laboratories and THL, and in 2011 the EUCAST standard was nationally adapted for AST.

For *S. aureus* the detection of the MRSA phenotype, and other possible acquired or adaptive resistance is of clinical importance[89]. The AST for *S. aureus* is usually performed according to standardized methodology (*e.g.* EUCAST, Clinical & Laboratory Standards Institute, CLSI) with Kirby-Bauer disk diffusion or MIC-methods (broth microdilution, gradient strip tests)[5,120].

Phenotypically, the 30 µg cefoxitin disc has been shown to reliably predict methicillin nonsusceptibility in clinical *S. aureus* isolates, while the oxacillin disc appears to present limited sensitivity[264]. The Finnish guideline does not mandate the use of further confirmation for MRSA detection, although molecular methods are widely used[220].

The antibiogram can be used as a phenotypic typing technique, as closely related isolates often carry and express similar susceptibility profile – especially in short term outbreak settings, or within-hospital in low-endemicity settings[179]. Antibiogram of an isolate is often readily available from diagnostic procedures and the discriminatory power has been reported to be comparable to ribotyping[239].

2.3.5 *spa* typing

spa typing, first suggested as a staphylococcal typing method to discriminate epidemic MRSA strains from nonepidemic in the 1990s, is based on the DNA sequencing of a variable region of the *spa* gene[265]. Typing result can be obtained with either Sanger sequencing or NGS methods[266]. The *spa* gene is approximately

2.15 kb long and encodes a cell wall anchored protein A. It can be divided into three distinct regions: the IgG Fc binding site, X region and C terminal domain. The X region includes the variable Xr part, consisting of variable number of 21–27 bp tandem repeats, which represents the area used for the discrimination of strains with *spa* typing[267].

While firstly the method compared the number of tandem repeats in Xr between strains, the discriminatory power of the method was increased with the identification of individual repeats, resulting in a strain specific repeat pattern, a ‘’fingerprint’’ unique for a strain[265,268]. The repeat pattern appears to maintain lineage specific genetic signal: *spa* typing has been shown to correctly classify *S. aureus* strains according to clonal lineages, with concordance values of 88% to whole genome DNA microarray[269]. More recently, concordance with WGS-based approaches in outbreak detection has been observed, although the resolution of *spa* typing is inferior to WGS for in-depth analysis[270]. The assembly of the repeat pattern from the short WGS reads can be challenging, which can result in suboptimal concordance between Sanger sequencing and WGS based *spa* typing[271]. This can be curbed partly in the bioinformatic processing or by using longer read length.

Establishment of an online service with uniform nomenclature enabled the identification of individual repeats as well as common repeat patterns (*spa* types) which in turn allowed interlaboratory reproducibility and comparison of typing results, leading to wider adaptation[268]. The server under Ridom GmBh (Würzburg, Germany) remains to date[272]. The spaserver database now includes 22,489 unique *spa* types from 194,920 strain records and 859 individual repeats (accessed 27th December, 2025). *spa* typing has been established as the routine MRSA typing method in Finland in 2009, with evidence of adequate concordance of typing results to the previously used PFGE[213].

Nontypeable (NT) strains in *spa* typing are uncommon (usually <2 % of human isolates), but may be more prevalent in ST398 (LA-MRSA)[273]. Issues in typing typically arise from genetic rearrangements in the IgG-binding region of the *spa* gene (primer-binding region); and technical issues[274]. Strains completely lacking a CWA protein A (frameshift mutation in the *spa* gene) and strains with significant deletions in the *spa* gene have also been described[275,276].

The expanding repertoire of different *spa* types, as well as arbitrary nomenclature of individual types sparked a need for a clustering tool for similar *spa* types. In 2007, Mellman *et al.*[277] described a based upon repeat pattern (BURP) algorithm for this purpose. The algorithm compares the *spa* type repeat patterns, calculating a cost for each ‘’evolutionary step’’ (repeat duplication and -excision, substitution, base-insertion and -deletion events) between the compared types. Cost value of ≤4 and excluding *spa* types shorter than 5 repeats provided an 87.1% concordance with a clustering based on whole-genome microarray technique.

2.3.6 Other genotyping methods

PFGE is a genotypic typing method, which results in strain specific, highly discriminatory genomic fingerprint pattern. It was considered the ''gold standard'' typing method until the early 2010s. The technique involves the digestion of target organism's DNA with specific, rare cutting (cleaves infrequently occurring DNA sequences, *Sma*I for *S. aureus*) restriction enzymes, followed by separation of resulting large genome fragments on a flat agarose gel in an alternating electric field[278]. By comparing the specific patterns, clonal relationship of strains can be assessed, as accumulating changes in the genome affect the band pattern.

Although PFGE is highly discriminatory for *S. aureus* strains, challenges associated with the method include difficulties in interlaboratory reproducibility and subjective interpretation leading to comparability issues with the results, as well as relatively rapid accumulation of genetic variation, resulting in unpredictable variation in the typing results[279]. This limits the use of PFGE for detection of clones for longer term epidemiological surveillance. The method is also laborious and technically complex, requiring expertise. For individual outbreak analyses, however, these limitations usually are not detrimental. Additionally, nontypeable (NT) strains such as the LA-MRSA lineage (ST398) have been reported and while *spa* typing has shown limited discrimination of the ST398 isolates, most of them exhibit either *spa* types t011, t108 or t034[280]. An adjusted PFGE protocol using a neoschizomer of *Sma*I (*Cfr9*I) has been successfully applied to reveal diversity within the ST398 strain[281].

MLST was first developed for *Neisseria meningitidis* and *Streptococcus pneumoniae,* for which the natural tendency for rapid horizontal gene exchange (recombination) is greater than *S. aureus,* and proves a disadvantage for PFGE[282,283]. The idea in MLST is to determine the nucleotide sequence of several conserved housekeeping genes, assigning an arbitrary number for each allele. The combination of the specific alleles forms a profile known as the MLST strain type (ST)[284]. The widely adapted MLST scheme for *S. aureus* was developed in the late 1990s at Oxford university and consists of seven loci (genes *arc, aroE, glpF, gmk, pta, tpi* and *yqiL*) present in virtually all MSSA and MRSA strains[285].

Similar to the BURP algorithm described for the clustering of *spa* types, clustering algorithm for STs have also emerged. The eBURST algorithm subdivides STs into exclusive groups with a user-defined number of identical alleles, and using a less stringent group definition, groups similar STs into clonal complexes (CCs). CCs are commonly used to describe most likely pattern of evolutionary descent, usually single or dual locus variants of the predicted founder[286]. Classification using CCs has been useful for large-scale epidemiological studies, as CCs appear to align well with genome-based phylogenetic branches: in a large study analysing 351 *S. aureus* food isolates representing 59 STs, 26 CCs and 252 core-genome MLST (cgMLST) complex types, the adjusted Wallace coefficient from cgMLST to MLST

was 1.0, which means that the MLST-based typing results could always be predicted from the WGS-based typing result[287]. Nowadays, a public, open-access MLST database with a large database of international isolates (48,247 *S. aureus* isolates in December, 2025) has been established including draft genomes and expanded allele profiles for more than 34,000 *S. aureus* isolates[288].

2.3.7 Whole-genome sequencing

Advances in the adaptation of genomics in microbiology during the last two decades has provided a surge of detailed information of about *S. aureus* as an infective micro-organism[289]. High-throughput sequencing methods have unlocked the ability to infer detailed genetic relationships of strains in community- and hospital outbreaks, providing a powerful tool for epidemiological research, surveillance and outbreak management[270].

The new sequencing technologies have unlocked new ways to analyse staphylococcal virulence and mechanisms of AMR, although predicting resistance phenotypes from WGS data has not been straightforward[290]. WGS based detection of antimicrobial resistance has been validated as a prospective method to predict phenotypic antimicrobial susceptibility[291]. By the detection of virulence factors and accumulating adaptations in recurrent infection, outcomes and risks of mortality could be predicted[292,293].

The most used WGS platforms include commercial platforms utilizing either short- or long-read sequencing lengths[294]. While platforms using short-read lengths have been widely adapted and offer excellent resolution, the developing long-read sequencing could offer sufficient discrimination, lower cost and faster typing in comparison with the short-read technology – at least for short term outbreak analysis[295].

While WGS remains as the most powerful tool to assess relatedness between isolates, factors related to the cost, analysis timeliness, and bioinformatic processing continue to hinder the adaptation of WGS in routine laboratory practice[290,296]. Phenotypic methods, such as MALDI-TOF and Fourier-transform infrared spectroscopy (FTIR) have been proposed, and largely applied, as quick and inexpensive tools to conduct routine diagnostics and rapid typing to reduce the need for extensive sequencing[238,240]. In Finland, WGS is offered for outbreak investigation of MRSA by THL and Tyks Laboratories[297].

2.3.8 Phenotypic methods

Phenotypic methods refer to strain identification techniques which are not based on the nucleotide sequences or genetic fingerprinting, but rather strain profiles based on biochemical characteristics (*e.g.* FTIR) or interaction with biological (*e.g.* phage

typing, serotyping) or chemical (*e.g.* antimicrobial susceptibility profile) agents. Phenotypic methods were fundamental before the development of molecular techniques, and some, like serotyping, are still widely used today (although not for *S. aureus*). Fourier transform infrared spectroscopy (FTIR) has its roots in analytical chemistry and microbiology of the 1980s and recently, new commercial adaptations offering straightforward analysis pipeline have made the technique more broadly available[238]. Phenotypic methods are often influenced by environmental conditions and require intense standardization of the laboratory protocol. They may depend on the (level of) expression of some genes and hence genotypic and phenotypic typing of the same dataset can result in discrepant partition.

2.3.8.1 Serotyping

Serotyping is based on the use of antisera (antibodies) against bacterial surface polysaccharides (capsular polysaccharide, CP). Although various (up to 13) different serotypes have been reported for *S. aureus*, approximately 75% of clinical strains express either type 5 or 8 CP, and the majority of the clinical NT strains react with a specific surface antigen, *i.e.* antigen 336, which is a teichoic-acid-like (as opposed to capsular-polysaccharide-like) poly(ribitol phosphate)-*N*-acetylglucosamine biopolymer[298,299]. Although non-encapsulated strains have been identified and associated with survival within leucocytes, causing chronic infection, the serotyping of *S. aureus* is of a limited clinical importance, has limited discriminatory power, is associated with technical complexity and high cost – and hence not performed in routine laboratory diagnostics or surveillance[300].

2.3.8.2 Fourier transform infrared spectroscopy

FTIR is a method traditionally used in biochemical analysis (molecular composition determination) of different sample types[238]. The technique was suggested for microbial strain typing already in the 1950s, but essential technical and software developments enabling more widespread use in microbiology happened in the 1990s[238,301]. The principle of the method is the measurement of absorption of infrared light passing through the sample material, a suspension of bacterial whole cell grown to the stationary phase under standard conditions[298]. The different functional groups, present mostly in the bacterial cell wall, absorb light at specific wavelengths, *i.e.* spectral windows[302]. Although FTIR has been applied to species identification, strain typing on the subspecies level appears as the most promising application, as the spectral window corresponding with polysaccharide structures (1,200–900 cm^{-1}) has shown success in predicting serotypes/-groups for many species, including *Salmonella*[303] and pneumococcus[304].

For *S. aureus,* FTIR has been mostly applied to serotyping and epidemiological strain identification. Amiali *et al.*[305] were able to differentiate a CA-MRSA strains from Canadian HA-MRSA lineages in a diverse dataset of 156 isolates with up to 98% accuracy. Grunert *et al.*[298] were able to apply FTIR for serotyping the most encountered capsular serotypes of *S. aureus* isolates (CP5, CP8 and NT) with 98.2% accuracy, by training an artificial neural network model[298]. The method is sensitive to small changes in the composition of the sample, and factor such as growth temperature and culture medium can have significant impact on the reproducibility of the typing result[306].

As FTIR based typing is based on the biochemical profile of mostly cell wall components, the best directional concordance was found in one study between FTIR and *cap* specific locus, while assignment of CCs with FTIR proved challenging – with the possible exception of CC45 and CC705[307]. However, the overall discriminatory power has been comparable to that of *spa* typing and PFGE, which makes FTIR an attractive rapid and cheap typing method for short term outbreak investigations[307,308]. More recently, high negative predictive value in outbreak analysis (meaning strains classified as unrelated to the outbreak) of FTIR for *S. aureus* has been observed, suggesting a role for the method as a cheap screening tool for isolates to be analysed further with high-resolution genotypic methods like WGS[309,310].

3 Aims

The primary objective of this thesis is to analyse the characteristics and temporal changes in strain types and AMR profiles of MRSA isolates collected in the hospital district of Southwest Finland from 2007 to 2016, with a focus on the prevalent *spa* t172 MRSA. Additionally, we aim to evaluate the performance and applicability of WGS and FTIR methods in local outbreak investigation.

The detailed aims of the present studies are to:

I. To understand the clonality, transmission and persistence of prevalent MRSA *spa* t172 - and to identify demographic risk factors for its acquisition in the hospital district of Southwest Finland, 2007–2016 (Study I)

II. To analyse the changing AMR pattern and strain types of MRSA isolates in the hospital district of Southwest Finland, 2007–2016. Additionally, to identify possible risk factors of AMR among MRSA isolates in this setting. (Study II)

III. Assess the potential of the FTIR method as a rapid strain typing tool for both MSSA and MRSA isolates, as well as the use of WGS and FTIR in outbreak investigations. (Studies I and III)

4 Materials and Methods

4.1 National MRSA surveillance and registers (I, II)

MRSA is listed as a notifiable disease in Finland by law (Communicable Diseases Act 2016/1227). Since 1995, clinical microbiology laboratories have notified all new MRSA findings from both screening and clinical specimens to the national infectious diseases register (tartuntatautirekisteri, NIDR), maintained by THL[4]. Laboratories also send corresponding isolates to a strain bank at THL; and THL performs *spa* typing to all new isolates[213].

Information on healthcare use and hospitalizations (both public and private providers nationally) are collected to Care Registers for Social Welfare and Health Care (hoitoilmoitusjärjestelmä, HILMO). The data is reported to and maintained by THL, as mandated by the Act on the National Institute for Health and Welfare 2008/668.

The public healthcare providers in Finland are currently organized under 21 Wellbeing Service Counties. Before 2023, the responsibility of organizing healthcare services was at municipalities, and joint municipal authorities. The hospital district (HD) of Southwest Finland was a joint municipal healthcare authority consisting of 28 member municipalities at the time of the studies I and II. Previous responsibilities of the municipalities and the HD were transferred to the Wellbeing Services County (WSC) of Southwest Finland (Varha) in the beginning of 2023.

4.2 Study populations and definitions (I, II, III)

Study populations for studies I and II included all new MRSA cases identified in the HD of Southwest Finland between 2007–2016 (catchment population of 478,500, representing 8.7% of the Finnish population in 2016)[9]. The catchment population of Tyks clinical microbiology laboratory included the county (2022 onward the WSC) of Southwest Finland with some 494,819 (10.7% of the Finnish population) residents by the end of 2024[190].

Data on the incidence rate of MRSA and hospital care were obtained from the NIDR and HILMO registers kept by THL, respectively. Data on demographics and

risk factors of cases were obtained from the electronic patient records and the regional register of hospital infections and carriers of resistant bacteria (SAI register, Neotide Oy, Vaasa, Finland) kept by the infection control unit of Turku university hospital (Tyks).

Demographic information of the cases, including age, sex, specimen type (screen or clinical specimen), immigration (including persons who were asylum seekers, refugees, residents of another country or other immigrants), intravenous drug use, livestock contact, healthcare worker (HCW) status and hospitalisation or long-term care in Finland within the last two years were collected. Information on travel, work or hospitalisation abroad within the previous one year was collected for all cases. Information on hospital care abroad within the previous two years was collected, when information on this was available. Since the change in the MRSA screening schema in 2015 to include hospital care abroad within one year of the MRSA identification, some cases with hospital care abroad within 1–2 years may have been missed in 2015–2016.

Cases were defined as HA-MRSA if they were hospitalized or stayed within a LTCF within the previous two years in Finland or one year abroad, preceding the MRSA identification. HA-MRSA cases also included HCWs and infants under 28 days of age. Cases were defined as CA-MRSA if the identification resulted from an outpatient setting or within two days after hospital admission, and the HA-MRSA criteria were not fulfilled.

Additionally, epidemiological information on the association of a case with an identified family cluster (FC) or a healthcare-associated outbreak (HAO) was included. FCs were defined as two or more cases living in the same household, and HAOs were defined as two or more cases staying in the same room or sharing a washing facility within a ward or a LTCF. HCWs attending the same wards or LTCFs were included in HAOs. Index cases of FCs and HAOs were defined as the case with the earliest MRSA identification date.

Isolates in the prospective material used in Study III included new MRSA identifications made in the clinical microbiology laboratory of Tyks during a six-month period in September 2023–February 2024. The collected information included data regarding the specimen type (blood, urine, superficial or deep swab), acquisition date and association to a HAO, as identified and reported by secure e-mail or via phone by the infection control unit of Tyks. Suspected outbreaks were defined as two or more cases identified in the same healthcare unit, ward or LTCF, and at least one of the cases in the suspected outbreak had to be sampled during the prospective collection period.

4.3 Ethical considerations

Studies I and II were approved by the Hospital District of Southwest Finland (T162/2016; J28/21). Study III was approved by the Wellbeing Services County of Southwest Finland (T2141/2023). Studies I and II were also approved by the Finnish Institute for Health and Welfare (THL336/6.02.00/2016; THL/319/5.05.00/2020). As stated in the act of the Medical Use of Human Organs, Tissues and Cells (101/2001) and Biobank Act (688/2012), and as confirmed by the Hospital District of Southwest Finland Research Ethics Committee, no ethical committee approvals or informed consent were needed for retrospective, register-based studies I and II. No ethical committee approval or informed consent were required for the laboratory-based Study III.

4.4 Statistical analyses (I, II)

Statistical analysis was conducted with SPSS Statistics v.18 software (IBM, Armonk, NY, USA). Categorical demographic variables were analysed with the Fisher's exact test. Mann–Whitney U test was used to compare median ages in Studies I and II. Trends for the annual proportion of resistant isolates to each included antimicrobial and the multi-resistant phenotype were analysed with the Cochran-Armitage test for trend. The presence of a trend was assessed separately for the periods 2007–2010 and 2011–2016 respectively, because of the change in the used AST standard from CLSI to EUCAST. The level of significance in studies I and II was two-tailed p of ≤ 0.05.

4.5 Laboratory methods

4.5.1 Bacterial isolates and AST (I, II, III)

In studies I, II and III, one MRSA isolate per patient was included. The MRSA isolates were stored in milk broth with 15% glycerol at -80˚C.

For all studies, *S. aureus* was detected from clinical specimens with standard methods including coagulase test and species identification with the VITEK2 (bioMérieux, Marcy-l'Étoile, France) and since 2011 MALDI-TOF MS (Bruker GmbH, Bremen, Germany) systems. MRSA was identified from screening specimens using a selective chromogenic agar (since 2007, Chromagar® MRSA II, BD, Franklin Lakes, NJ, USA) or an enrichment broth (multiple suppliers, since 2018: eMRSA, Copan Diagnostics, Murrieta, CA, USA). MRSA was identified with either cefoxitin disk diffusion (Oxoid, Thermo Fisher Scientific, Waltham, MA, USA) test in AST according to the CLSI standard, and from 2011 onward the

EUCAST standard, or directly from clinical specimen with the easyplex MRSA kit (Amplex diagnostics GmbH, Gars, Germany). Quality control strains, necessitated by the CLSI and EUCAST standard (*S. aureus* ATCC29213), were used throughout the study period. The distribution of inhibition zone diameters in cefoxitin disk diffusion test remained constantly at the level determined by EUCAST standard (target 27 mm, range 24–30 mm).

For studies I and II, the presence of *mecA* (from 2014 onward also *mecC*) was confirmed with a commercial kit (Evigene MRSA kit, Statens Serum Institut, Copenhagen, Denmark until 2010, Xpert MRSA kits, Cepheid, Sunnyvale, CA, USA until 2016). For Study II, the phenotypic antimicrobial susceptibility was determined with disk diffusion for ten clinically relevant antimicrobials according to the CLSI standard, and from 2011 onward the EUCAST standard: cefoxitin, clindamycin (CLI), erythromycin (ERY), fusidic acid (FUS), gentamicin (GEN), levofloxacin (LVX), rifampicin (RIF), tetracycline (TET) and trimethoprim/sulfamethoxazole (SXT). A gradient strip test (E-test, bioMérieux, Marcy-l'Étoile, France) was used for vancomycin (VAN) AST.

MRSA is considered a MDR organism by virtue[311]. To analyse the co-resistance patterns of MRSA isolates in Study II, we defined MRSA isolates as multi-resistant when resistance was observed in two or more of the tested non-β-lactam antibiotics. This was done due to the suspected rarity of the extensively drug-resistant phenotype (non-susceptibility to 1 or more antimicrobials in all but 2 or more categories of antimicrobials tested for) in Southwest Finland[311]. Also, isolates assigned to the EUCAST category 'susceptible, increased exposure' or CLSI category 'intermediate' were interpreted as susceptible in the statistical demographic and trend analyses – to avoid overestimation of resistance.

For Study III, a prospective strain collection for the performance evaluation of FTIR method was conducted in the Tyks Clinical microbiology laboratory during a period from September 2023 to February 2024. The collection included both MSSA and MRSA isolates: a) all new *S. aureus* isolates from diagnostic blood cultures and b) all new MRSA isolates from all diagnostic clinical (blood, superficial and deep skin swabs, urine) and screening specimens.

4.5.2 Whole genome sequencing (I, III)

For WGS, *S. aureus* isolates were inoculated from storage tubes and cultured overnight on TSA agar supplemented with 5 % horse blood (Becton Dickinson, New Jersey, USA). The DNA was extracted with MagAttract HMW DNA kit (Qiagen, Hilden, Germany). Libraries were normalized using the Qubit fluorometer (Invitrogen, Waltham, MA, USA) and 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). Sequencing libraries were prepared with the Nextera XT DNA sample

preparation kit, and WGS performed with the MiSeq (Study I) and NextSeq 2000 (Study III) systems using 250 bp (Study I) and 300 bp (Study III) paired-end reads (Illumina, San Diego, CA, USA).

In Study I, SeqSphere+ v.7.2.3 (Ridom Gmbh, Würzburg, Germany) was used for the analysis of the WGS reads, including data quality control (FastQC) and adapter trimming. *De novo* assembly was generated with the implemented SKESA v.2.3 software[312]. A default public task template for *S. aureus* with 1861 targets was used for MLST and core genome MLST (cgMLST) allele calling, and isolates with <95% of targets passing the quality control were excluded[313]. A sample-centric single-nucleotide variant (SNV) search was used to generate a distance matrix of the isolates, and a minimum-spanning tree (MST) called as implemented in the SeqSphere+ software. Pairwise ignoring of missing values option was used and clustering cutoff set to a conservative 15 SNVs to outline closely related isolates[314]. The prescence of an SCC*mec* element and their types were analysed with a web-based tool SCCmecFinder v.1.2, assuming the cassette type with the best match (homology) when missing genes were observed[130].

In Study III, the sequences were assembled by mapping to a reference genome (GenBank: CP014791.1) using the CLC Microbial Genomics Module v.24.1.1 software (Qiagen Digital Insights, Aarhus, Denmark). A maximum-likelihood phylogeny and an SNV matrix were inferred using standard parameter (Jukes Cantor substitution model, ignoring multi-nucleotide variants (MNVs), substitution rate variation) on the SNP Tree tool[315]. Sequence types (STs) and *spa* types were called from the reads using a k-mer based tool integrated in the CLC software. The same clustering threshold of 15 SNVs as in Study I was used[314]. Additionally, isolates with 16–49 SNVs were considered as related while isolates with >49 SNVs were considered unrelated[316]. IToL software was used to visualize and annotate phylogenomic data[317]. All sequence data have been deposited to the NCBI Sequence Read Archive under BioProject accession no. PRJNA826681 (Study I) and PRJNA1372986 (Study III).

4.5.3 *spa* typing (I, II)

spa typing was conducted as previously described[268,272]. For Studies I and II, MRSA isolates were *spa* typed in-house at the University of Turku, Institute of Biomedicine for isolates from 2007–2008, and in THL for isolates from 2009–2016, when routine *spa* typing was adapted for national surveillance[213,318]. For Study III, *spa* typing of MRSA isolates was performed by THL, and the typing of MSSA isolates was performed at the University of Turku, Institute of Biomedicine.

In the University of Turku, DNA was extracted with the NucleoSpin Microbial DNA kit (Macherey-Nagel, Düren, Germany). A PCR was applied to amplify the staphylococcal *spa* repeat region in a reaction with HPLC-cleaned primers: spa-

1113f (5'- TAA AGA CGA TCC TTC GGT GAG C -3') and spa-1514r (5'CAG CAG TAG TGC CGT TTG CTT -3'). The product was inspected with gel electrophoresis, and Sanger sequenced. The resulting *spa* gene sequence was analysed with the Ridom StaphType™ software (Ridom GmbH, Würzburg, Germany) in Studies I and II - and with the web-based tools spaTyper and Ridom *spa* server for Study III[272,319].

BURP algorithm was used to cluster similar *spa* types (see chapter 2.3.5). As proposed by Mellman *et al.*[233], *spa* types shorter than five repeats were excluded, and strains with cost values of less than or equal to four were clustered to *spa* clonal complexes (spa-CCs, see chapter 2.3.5). The *spa* type with the highest sum of cost values within a cluster was defined as founder, and each spa-CC is named according to its founder.

4.5.4 Fourier transform infrared spectroscopy (III)

4.5.4.1 Laboratory workflow (III)

FTIR analysis pipeline on the IR Biotyper® platform (Bruker Daltonics GmbH & Co. KG, Bremen, Germany) was set up in the Tyks clinical microbiology laboratory. *S. aureus* strains were cultured from the 15% glycerol storage tubes, stored in -80˚C, or from the primary diagnostic culture. With a 1 µl plastic loop, the isolates were inoculated to Müller-Hinton (MH) agar plates (Becton Dickinson, New Jersey, USA) and cultured under standard conditions: 18 hours in 35˚C, 5% CO_2. The isolates were subcultured to MH plates following the initial incubation, and the standard incubation repeated. The subculture was used for spectral acquisition.

The spectral acquisition workflow was conducted according to the manufacturer's instructions with a slight modification in reagent volume (to ease suspension homogenisation step). Briefly, a 1 µl plastic loop was used to carefully collect two loopfuls of bacterial biomass from the MH culture plate. The biomass was then suspended into 100 µl of 70% (v/v) ethanol and vortexed vigorously until complete homogenisation of the suspension was observed. 100 µl of LC/MS grade water was added, and the suspension was again briefly vortexed. 15 µl of the suspension was pipetted to sample spots of the IR Biotyper® 96-well silicon microtiter plate. The plate was dried for 10 to 15 minutes in room temperature and visually inspected for signs of under- or overdrying before spectral acquisition. Each sample was pipetted to four different spots resulting in the acquisition of four technical replicate spectra per isolate per run, of which we aimed to acquire three technical replicate spectra passing the preprogrammed quality control parameters. These parameters ensured within-range turbidity of the initial suspension, as well as the lack of noise in the spectral acquisition signal, which could result from suboptimal homogenisation or moisture of the specimen. The same protocol for each sample was repeated from initial inoculation on

at least two different days to acquire two biological replicates, resulting in a total of six spectra per sample for downstream analysis. Two *E. coli* reference strains (IRTS 1 and IRTS 2, Bruker) were used as a control in each run and the ATCC-25923 strain was used to validate between runs.

4.5.4.2 Spectral analysis pipeline (III)

In the spectral analysis phase, the default acquisition range of wavenumbers 1,300–800 cm^{-1} was used. Preprocessing of the spectral data, including vector normalization, smoothing with the Savitzky-Golay filter and the calculation of second derivatives, was automatically performed by the OPUS (v4) software implemented in the IR Biotyper® platform software. The spectra were exported as csv-files with 500 data points corresponding spectral coordinates (1 data point per wavenumber value) for further analysis with the Quasar (v1.11.1) and PAST (v5.2) softwares[320,321]. The isolate metadata and spectral data generated in this study were deposited in Zenodo under DOI: 10.5281/zenodo.17802985.

For unsupervised analysis of all isolates and the outbreak isolates, *i.e.* clustering without previously defined groups, the Euclidean method was applied to calculate distance values between the spectra and a distance matrix acquired (**Figure 6**). From the distance matrix, hierarchical clustering analysis (HCA with Ward's linkage algorithm) and principal component analysis (PCA) with a target to explain 99% of the variance in the dataset, could be performed. The result of the HCA was visualised and analysed as a dendrogram, while the PCA was further analysed in a two-dimensional scatter plot using PC1 and PC2 as the axes. For a supervised analysis of the outbreak isolates, the PAST v.5.2 software was used to conduct a linear discriminant analysis (LDA)[321]. Using the extracted principal components, LDA was calculated using the isolate name as grouping identifier. The LDA centroid axes (mean value of the the six LDA axis per isolate) were used in HCA (Ward's linkage algorithm) to infer a dendrogram of the outbreak isolates.

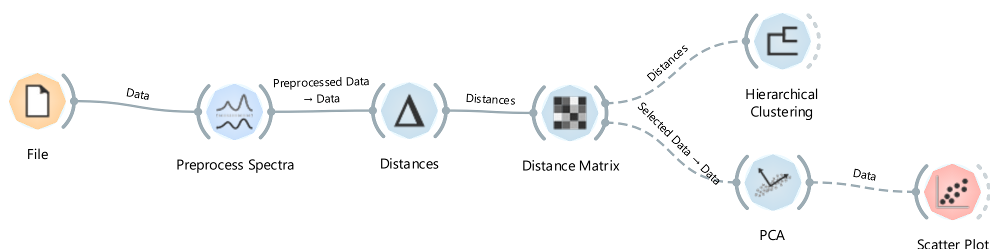

Figure 6. Example of the spectral analysis pipeline as a step by step process by widgets implemented in Quasar (v1.11.1) software[312]. The "Preprocess spectra" widget was used to visually inspect the imported data. A screenshot from the Quasar software.

5 Results

5.1 MRSA cases and strain types, 2007–2016 (I, II)

Within our 10-year retrospective, population-based study, 983 MRSA cases were identified from electronic patient records in the Hospital District of Southwest Finland, 2007–2016[9]. In Study I, isolates without a recorded *spa* typing result or a stored, available isolate for *spa* typing, were discarded from the dataset (7 isolates, n=976). In Study II, isolates without previously documented microbiological confirmation of the MRSA phenotype, or available isolate for AST, were discarded from the dataset (12 isolates, n=971). Discrepancy of the case count between retrospective registry sources remained inexplicable.

All available isolates with microbiological confirmation of the MRSA phenotype, were *spa* typed, and similar *spa* types were clustered (spa-CC clusters) with the BURP algorithm (see chapter 4.5.3). Altogether 173 different *spa* types, and 17 nontypeable isolates were detected. Within 15 *spa* types, ten or more isolates were detected, accounting for 71.2% of all isolates. Among 59 *spa* types, two to eight isolates were found (18.6% of all isolates) and 99 *spa* types were singletons (10.2% of all isolates). As per the clustering parameters, the *spa* types were clustered to 13 spa-CCs and 13 singletons; 15 *spa* types were excluded from the clustering and 17 isolates remained nontypeable (**Table 2**).

The six largest spa-CCs (172, 002, 790, 008, 012 and 4137/267) collectively accounted for 795 (81.9%) of all 971 isolates, while among single *spa* types, t172 was the most abundant (n=237, 24.4%), followed by t008 (n=73, 7.5%), t002 (n=51, 5.3%) and t032 (n=40, 4.1%). In the BURP clustering, 13 isolates (1.3% of all) remained singletons and 54 (5.6% of all) isolates representing the 15 *spa* types shorter than five repeats were excluded from the analysis.

Table 2. Distribution of MRSA isolates (n=971) to spa-CC clusters and *spa* types - and the number of multi-resistant isolates by *spa* type, Hospital District of Southwest Finland, 2007–2016. Adapted from Study II.

Spa-CCs (% of all isolates)	***Spa* type: n isolates (% of all isolates)**	**Multi-resistant MRSA isolates by *spa* type: n isolates (% of isolates with the same *spa* type)**
spa-CC 172: 12 different *spa* types 273 (28.1) isolates	t172: 237 (24.4), t437: 16 (1.6), t2976: 6 (0.6), t1146: 4 (0.4), t3527: 2 (0.2), t2380: 2 (0.2), t216: 1 (0.1), t10053: 1 (0.1), t1126: 1 (0.1), t13749: 1 (0.1), t441: 1 (0.1), t7726: 1 (0.1)	t437: 13 (81.3), t172: 3 (1.3), t3527: 1 (50.0), t216: 1 (100), t10053: 1 (100)
spa-CC 002: 25 different *spa* types 143 (14.7) isolates	t002: 51 (5.3), t688: 37 (38.1), t067: 15 (1.5), t010: 5 (0.5), t105: 4 (0.4), t5150: 4 (0.4), t548: 3 (0.3), t653: 2 (0.2), t003: 2 (0.2), t242: 2 (0.2), t442: 2 (0.2), t045: 2 (0.2), t311: 2 (0.2), t2460: 1 (0.1), t315: 1 (0.1), t443: 1 (0.1), t9231: 1 (0.1), t9408: 1 (0.1), t1818: 1 (0.1), t1062: 1 (0.1), t088: 1 (0.1), t1567: 1 (0.1), t6356: 1 (0.1), t2249: 1 (0.1), t306: 1 (0.1)	t002: 14 (27.5), t688: 7 (18.9), t067: 3 (20.0), t548: 3 (100), t653: 2 (100), t003: 2 (100), t242: 2 (100), t442: 2 (100), t010: 1 (20.0), t105: 1 (25.0), t045: 1 (50.0), t2460: 1 (100), t315: 1 (100), t443: 1 (100), t9231: 1 (100), t9408: 1 (100)
Spa-CC 790: 25 different *spa types* 122 (12.6) isolates	t032: 40 (4.1), t223: 38 (3.9), t005: 8 (0.8), t790: 5 (0.5), t020: 4 (0.4), t891: 3 (0.3), t5983: 2 (0.2), t910: 2 (0.2), t379: 2 (0.2), t670: 2 (0.2), t310: 2 (0.2), t9053: 1 (0.1), t852: 1 (0.1), t515: 1 (0.1), t022: 1 (0.1), t1302: 1 (0.1), t557: 1 (0.1), t5815: 1 (0.1), t608: 1 (0.1), t625: 1 (0.1), t15108: 1 (0.1), t2033: 1 (0.1), t3689: 1 (0.1), t449: 1 (0.1), t8178: 1 (0.1)	t223: 6 (15.8), t032: 5 (12.5), t005 (50.0), t020: 2 (50.0), t5983: 2 (100), t910: 2 (100), t9053: 1 (100), t852: 1 (100), t515: 1 (100)
Spa-CC 008: 16 different *spa* types 118 (12.2) isolates	t008: 73 (7.5), t304: 22 (2.3), t334 4 (0.4), t064 3 (0.3), t121: 2 (0.2), t190: 2 (0.2), t024: 2 (0.2), t622: 2 (0.2), t5071: 1 (0.1), t1635: 1 (0.1), t1257: 1 (0.1), t207: 1 (0.1), t11474: 1 (0.1), t13375: 1 (0.1), t1767, t919: 1 (0.1)	t008: 22 (30.1), t304: 5 (22.7), t064: 3 (100), t334: 1 (25.0), t121: 2 (100), t190: 1 (50.0), t5071 (100), t1635: 1 (100), t1257: 1 (100)
Spa-CC 012: 20 different *spa* types 81 (8.3) isolates	t019: 24 (2.5), t034: 20 (2.1), t021: 5 (0.5), t037: 4 (0.4), t2741: 3 (0.3), t108: 3 (0.3), t012: 3 (0.3), t2582: 2 (0.2), t363: 2 (0.2), t632: 2 (0.2), t1642: 2 (0.2), t253: 2 (0.2), t5308: 2 (0.2), t011: 1 (0.1), t1250: 1 (0.1), t4912: 1 (0.1), t1752: 1 (0.1), t300: 1 (0.1), t3593: 1 (0.1), t404: 1 (0.1)	t034: 18 (90.0), t021: 4 (80.0), t037: 4 (100), t2741: 3 (100), t019: 2 (8.3), t2582: 2 (100), t363: 2 (100), t632: 1 (50.0), t108: 1 (33.3), t011: 1 (100), t1250: 1 (100)
Spa-CC 4173/267: 13 different *spa* types 58 (6.0) isolates	t044: 34 (3.5), t189: 5 (0.5), t657: 4 (0.4), t345: 3 (0.3), t267: 3 (0.3), t2802: 2 (0.2), t5755: 1 (0.1), t10863: 10 (0.1), t5019: 1 (0.1), t9873: 1 (0.1), t131: 1 (0.1), t4173: 1 (0.1), t521: 1 (0.1)	t044: 23 (67.6), t189: 5 (100), t657: 3 (75.0), t345: 3 (100), t5755: 1 (100)

Spa-CCs (% of all isolates)	*Spa* type: n isolates (% of all isolates)	Multi-resistant MRSA isolates by *spa* type: n isolates (% of isolates with the same *spa* type)
Spa-CC 065: 3 different *spa* types 31 (3.2) isolates	t040: 29 (3.0), t180: 1 (0.1), t065: 1 (0.1)	t180: 1 (100)
Spa-CC 321/127: 3 different *spa* types 29 (3.0) isolates	t127: 26 (2.7), t174: 2 (0.2), t321: 1 (0.1)	t127: 16 (61.5), t174: 2 (100), t321: 1 (100)
Spa-CC 015: 11 different *spa* types 21 (2.2) isolates	t1081: 5 (0.5), t015: 3 (0.3), t1359: 2 (0.2), t102: 2 (0.2), t116: 2 (0.2), t620: 2 (0.2), t050: 1 (0.1), t2334: 1 (0.1), t4999: 1 (0.1), t6926: 1 (0.1), t728 (0.1)	t1359: 2 (100), t1081: 1 (20.0)
Spa-CC 186: 10 different *spa* types 16 (1.6) isolates	t690: 4 (0.4), t2894: 4 (0.4), t1855: 1 (0.1), t1603: 1 (0.1), t1816: 1 (0.1), t186: 1 (0.1), t1339: 1 (0.1), t16017: 1 (0.1), t325: 1 (0.1), t786: 1 (0.1)	t690: 2 (50.0), t1855: 1 (100), t1603: 1 (100), t1816: 1 (100)
Spa-CC 160 3 different *spa* types 6 (0.6) isolates	t156: 4 (0.4), t160: 1 (0.1), t771: 1 (0.1)	t160: 1 (100)
*No founder 1&2** 4 different *spa* types 6 (0.3) isolates	1: t324: 2 (0.2), t126: 1 (0.1) 2: t4000: 2 (0.2), t3070: 1 (100)	-
Singletons 13 different *spa* types 13 (1.3) isolates	t525: 1 (0.1), t4690: 1 (0.1), t435: 1 (0.1), t3487: 1 (0.1), t370: 1 (0.1), t6675: 1 (0.1), t1419: 1 (0.1), t355: 1 (0.1), t7470: 1 (0.1), t11717: 1 (0.1), t14228: 1 (0.1), t202: 1 (0.1), t346: 1 (0.1)	t525: 1 (100), t4690: 1 (100), t435: 1 (100)
*Excluded*** 15 different *spa* types 54 (5.6) isolates	t386: 12 (1.2), t026: 7 (0.7), t362: 6 (0.6), t1304: 1 (0.1), t13297: 1 (0.1), t233: 1 (0.1), t693: 1 (0.1), t777: 1 (0.1), t1109: 1 (0.1), t15730: 1 (0.1), t2884: 1 (0.1), t390: 1 (0.1), t529: 1 (0.1), t586: 1 (0.1), t994: 1 (0.1), nontypeable: 17 (1.8)	t386: 12 (100), t026: 2 (28.6), t1304: 1 (100), nontypeable: 5 (29.4)

*No founder can be determined for spa-CCs with two *spa* types. **Types with four repeats or less were excluded from clustering

The relative proportions of *spa* types and spa-CCs changed over time during the study period (**Figure 7**). The diversity of the MRSA strain types increased towards the end of the study period, marked by a decreasing proportion of spa-CCs 172 and 002, while the proportions of spa-CCs 012, 4173/267, small spa-CCs and singletons were increasing.

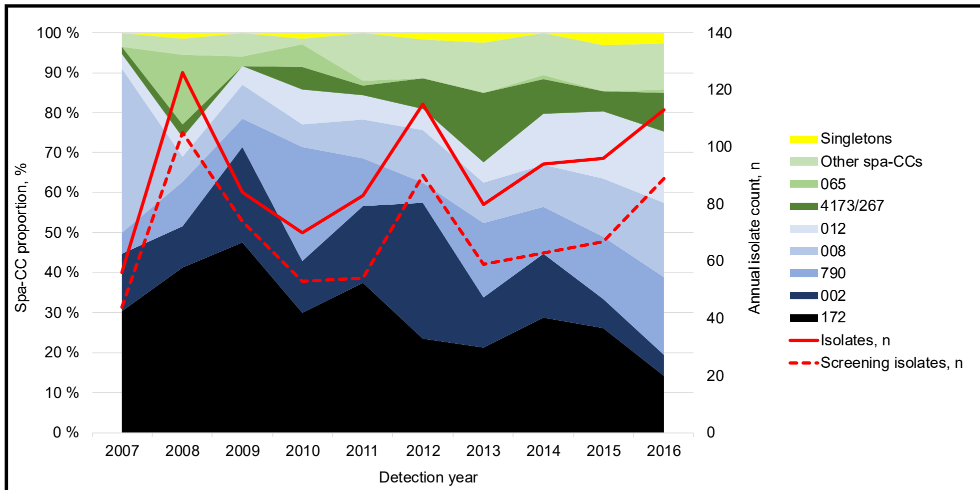

Figure 7. Annual proportion (%) of spa-CC clusters with total number of isolates and isolates from screening specimens shown in the secondary axis, Hospital District of Southwest Finland, 2007–2016. Adapted from Study II.

5.2 Demographic and epidemiological characteristics of the *spa* t172 MRSA cases, 2007–2016 (I)

In Study I, the risk factors and transmission of the prevalent t172 MRSA strain in Southwest Finland were examined. A total of 237 t172 isolates, representing 24.3% of 976 MRSA isolates detected in the HD of Southwest Finland, 2007–2016, were identified and linked with 170 HA-MRSA and 67 CA-MRSA cases (by our epidemiological definition, **Table 3**). Among the HA-MRSA cases, 74 cases were associated with healthcare associated outbreaks (HAOs), 51 cases with family clusters (FCs) and 45 cases were defined as other HA-MRSA cases. Among the CA-MRSA cases, 50 cases were associated with FCs, and 17 cases were defined as sporadic, without known links to FCs or to healthcare.

Table 3. Demographic and epidemiological characteristics and risk factors associated with the *spa* t172 MRSA strain, HD of Southwest Finland, 2007–2016. Adapted from Study I.

VARIABLE	CASES WITH *SPA* t172 STRAIN, N (%)	OTHER CASES, N (%)	*p*[d]
CASE COUNT	237 (24.3)	739 (75.7)	N/A
MEDIAN AGE, YEARS, IQR	58.4, 34.2–82.6	39.6, 17.1–62.2	**<0.001**
MALE SEX	116 (48.9)	326 (44.1)	0.203
SCREENING SPECIMEN	177 (74.7)	525 (71.0)	0.319
IMMIGRANT STATUS[a]	8 (3.4)	201 (27.2)	**<0.001**
TRAVEL OR WORK ABROAD[b]	5 (2.1)	83 (11.2)	**<0.001**
LIVESTOCK CONTACT	0 (0)	22 (3.0)	**0.004**
INTRAVENOUS DRUG USE	1 (0.4)	5 (0.7)	N/A
LINK TO FC	101 (42.6)	220 (29.8)	**<0.001**
CA-MRSA*	67 (28.3)	240 (32.5)	0.260
SPORADIC CASE	17 (7.2)	126 (17.1)	**<0.001**
HA-MRSA*	170 (71.7)	499 (67.5)	0.260
LONG-TERM CARE RESIDENCE	55 (23.2)	120 (16.2)	**0.019**
HEALTHCARE WORKER STATUS	15 (6.3)	54 (7.3)	0.494
HOSPITAL CARE ABROAD[c]	12 (5.1)	143 (19.4)	**<0.001**
LINK TO HAO	74 (31.2)	149 (20.2)	**0.001**

[a]Asylum seeker, refugee, resident of another country or other immigrant status [b]Within the previous year, where information available [c]Within the previous two years, where information available [d]Fisher's exact test or Mann-Whitney U test where appropriate, statistical significance $p \leq 0.05$, significant values bolded. IQR interquartile range, CA-/HA-MRSA* community-associated-/healthcare-associated methicillin-resistant *S. aureus* (see definition p. 47), FC family cluster, HAO healthcare associated outbreak

The MRSA cases with the *spa* t172 strain were associated with higher median age than other cases (58.4 vs 49.6 years, *p*<0.001), whereas no differences were observed in terms of sex, healthcare worker status or specimen type. Although the t172 cases were not clearly associated with the either defined HA- or CA-MRSA groups, they were associated with the FCs, HAOs and long-term care residence,

suggesting active screening played an important role for the detection of this strain type. On the other hand, the non-t172 MRSA cases were associated with links to foreign countries, which is reflected by their association to immigrant status, travel or work and hospital care abroad. Additionally, intravenous drug use, livestock contact, and sporadic case definition were more abundant among the non-t172 cases.

5.3 WGS cluster analysis of the MRSA *spa* t172 isolates, 2007–2016 (I)

The MRSA t172 cases were involved in multiple MRSA outbreaks, as identified and reported by the Tyks Infection control unit: among the outbreaks (a total of 129 FCs and 26 HAOs), 2007–2016, t172 isolates were involved in 12 (46.2%) HAOs and 39 (30.2%) FCs. Those outbreaks, where the index case was caused by a t172 strain, were identified and selected for exploratory analysis with WGS (total n=64). These included 9 HAOs and 38 FCs. Additionally, all sporadic (no identified epidemiological links to other MRSA cases or to healthcare) t172 cases (n=17) were included. Three HAOs and one FC, where t172 was involved - but was not isolated from the index case were excluded. One case was excluded due to missing specimen (sporadic case, CA-MRSA group, 2016) and one due to low-quality sequence data (FC index case, HA-MRA group, 2013) making the total sample count for further analysis 62. An average of 125-fold coverage (range 75–183) and N50 of 79,280 bp were observed in WGS.

Sixty isolates showed ST375 and two were single-locus variants of ST375: ST59 and ST5428. No discrepancy was observed between conventional and WGS based *spa* typing. From the WGS reads, predicted SCC*mec* types were IV(2B) for sixty isolates, and V(5C2&5) for two isolates, namely SWF_2 (nonclustered) and SWF_13 (WGS cluster 9, **Figure 8**).

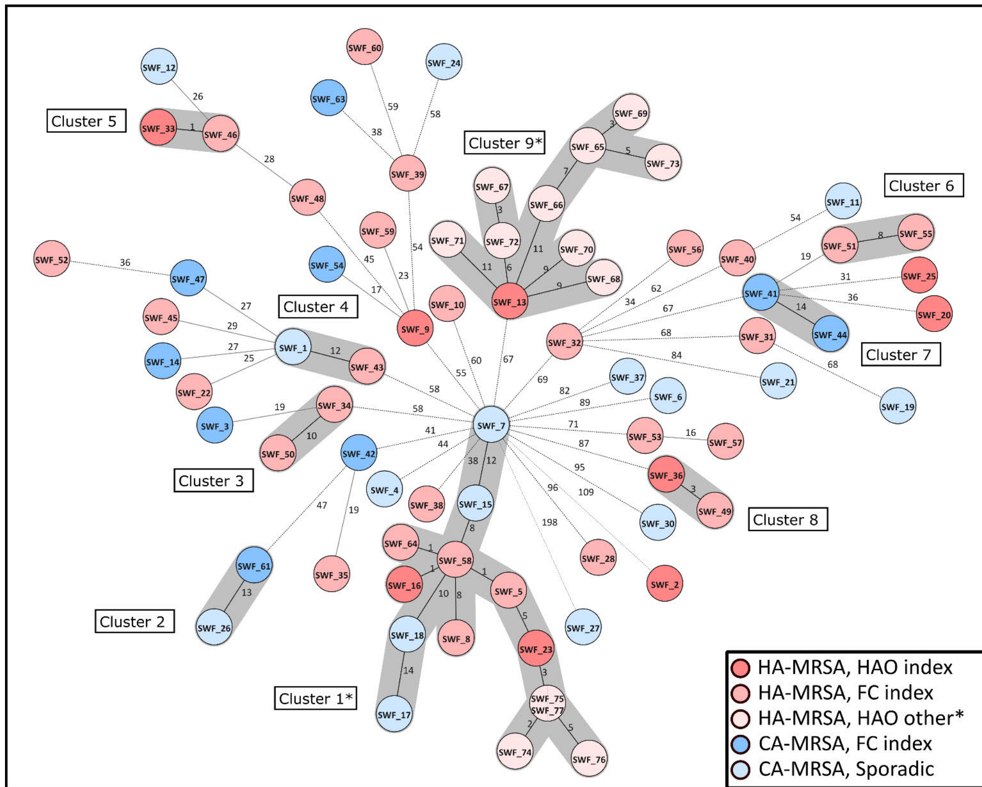

Figure 8. Minimum spanning tree of 75 MRSA *spa* t172 isolates, Hospital District of Southwest Finland, 2007–2016. Each node represents one or two isolates, distance between nodes measured by SNV differences in 1861 core genome alleles. 62 isolates represent index cases from healthcare-associated outbreaks (HAO, n=9) and family clusters (FC, n=37) and sporadic cases (n=16). 13 isolates in clusters 1 and 9, indicated with an asterisk (HA-MRSA, HAO other*), represent cases from known HAO investigations. MST generated with a cgMLST scheme including 1861 targets (SeqSphere+ v.7.2.3, Ridom GmbH, Würzburg, Germany). Adapted from Study I.

In the cgMLST analysis, the pairwise median SNV distance was 75 (range 1–242). With the clustering threshold of 15 SNVs in the cgMLST scheme, eight distinct clusters (Clusters 1 to 8) were detected comprising 24 (38.7%) isolates while 38 (61.3%) isolates were not clustered (**Figure 8**). The cluster 9 and an extension of cluster 1 (HA-MRSA case, HAO other* in **Figure 8**) comprised of 13 additional t172 MRSA isolates not included in the original dataset. These 13 isolates were sequenced as part of two HAO investigations (index cases SWF_13 and SWF_23) conducted by the Tyks Infection control unit, and were used as technical controls and included here for comparison. As seen in cluster 1, the scope of the outbreak was greater than identified in the initial investigation. Cluster 9, on the other hand, only included isolates which were identified in the initial investigation.

Cluster 1 identified in the cgMLST analysis, incorporated ten isolates from the strain selection, and four additional isolates from HAO investigations. The clustered isolates represented variable epidemiological backgrounds: four isolates from sporadic CA-MRSA cases, two isolates from HAO index cases and four isolates from HA-MRSA cases, which were also FC indexes. Isolates in cluster 1 spanned multiple years from 2009 to 2013 (**Figure 9**). The four additional isolates from a HAO investigation, clustered together with the original cluster 1. These additional isolates were detected in 2016, indicating that ongoing transmission of this subclone in the community and healthcare institutions, preceded the detection of the cluster by at least 7 years. However, suspicion of linkage between three out of four of the HA-MRSA FC index cases to the first HAO index case (SWF_16) in the cluster 1 pre-existed – and were only confirmed here with WGS. Nonetheless, the WGS analysis revealed previously unsuspected interlinkage of the four sporadic cases, one FC index case and the link between the two HAOs included in cluster 1.

Clusters 2 to 8 comprised two isolates each. All clusters involved a FC index case, and clusters 3, 6 and 7 included only FC index cases. Clusters 5 and 8 revealed a link between a HAO index case and a FC index case. Other than cluster 5, there was no previously suspected epidemiological links between the clustered isolates in clusters 2 to 8. Isolation dates suggested transmission direction from healthcare to households in clusters 1, 5 and 8 (**Figure 9**).

The nonclustered isolates were separated from other isolates by 16–198 pairwise SNVs. Isolates close to the clustering threshold (16 to 19 SNVs) could be observed and would have expanded two existing clusters (cluster 3 and clusters 6 and 7) and generated three new clusters (isolates SWF_53 and SWF_57, isolates SWF_9 and SWF_54, isolates SWF_35 and SWF_42, **Figure 8**). Otherwise, the distinction between small clusters and singleton isolates was clear (≥25 SNVs), although only two isolates (SWF_2 and SWF_27) differed by more than 100 pairwise SNVs from their closest isolate.

The nonclustered isolates represented 10 sporadic, 23 FC and 5 HAO index cases. These isolates were reported throughout the study period. Based on the epidemiological data, one FC index case (SWF_52) was linked to a HAO (index SWF_9), but interestingly, based on the WGS analysis, they were not closely related (pairwise distance 72 SNVs) refuting probable transmission. No other apparent epidemiological links were refuted in the WGS analysis.

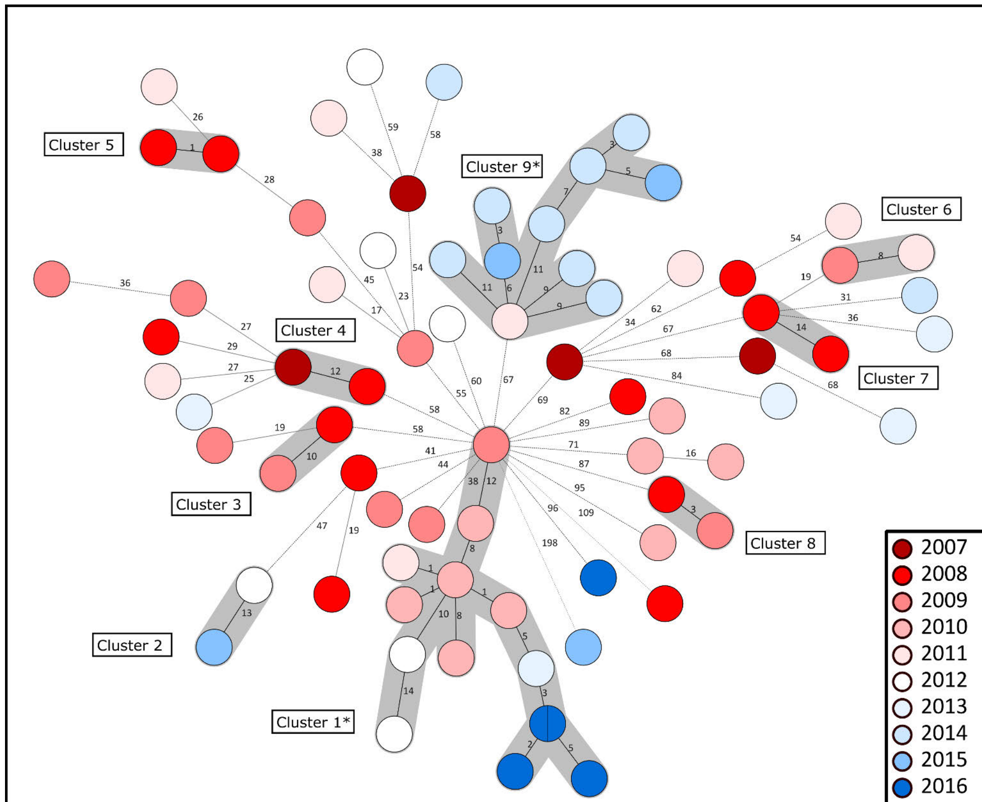

Figure 9. Minimum spanning tree of 75 MRSA *spa* t172 isolates, Hospital District of Southwest Finland, 2007–2016. Isolate detection year indicated in the legend. Adapted from Study I.

5.4 AMR trends of MRSA isolates, 2007–2016 (II)

In Study II, the pattern and changes in AMR of MRSA isolates was analysed through 2007–2016. Because of the change in the applied AST standard from CLSI to EUCAST (2011 onward), the trends in the AMR patterns were analysed for 2007–2010 and 2011–2016 separately. The annual number of MRSA isolates varied, with an annual median of 89 isolates (range: 56–126 isolates/year). The number of screening specimens was 698 (71.9%) in total, representing a median of 65 isolates annually (range 44–105 isolates/year); the proportion of screening specimens was highest in 2009 (74/90 isolates, 82.2%) and lowest in 2014 (63/99 isolates, 63.6%) (**Figure 7**).

Before the year 2011, increasing trends of AMR were observed for trimethoprim-sulfamethoxazole, (1.7 to 11.1%, p=0.03) and tetracycline (5.2 to 16.7%, p=0.005, **Figure 10**). After 2011 and by the end of the study period in 2016, the increasing trend of tetracycline resistance continued (16.7 to 32.0%, p<0.001).

The proportion of trimethoprim-sulfamethoxazole, resistant isolates increased until 2015, reaching 15.7%, but decreased to 2.4% in 2016. In addition to tetracycline, significant increasing resistance trends were observed to clindamycin (14.7 to 31.5%, $p<0.001$) and erythromycin (19.4 to 35.4%, $p<0.001$), between 2011–2016 respectively. Proportion of levofloxacin resistance was the highest among all tested antimicrobials in the 2007–2011 period (range: 14.6–25.9%), peaking in 2014 (26.0%), and followed by a decreasing trend of resistance towards 2016 (13.8%, $p=0.02$) in the latter period of 2011–2016.

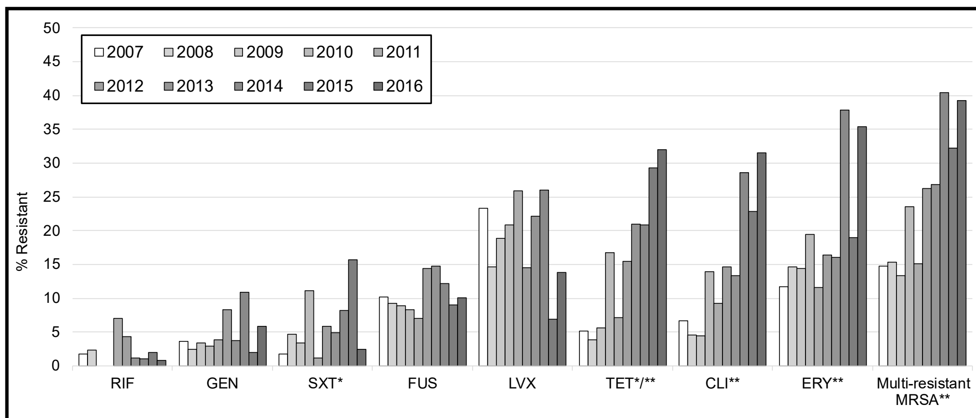

Figure 10. Annual proportion of antimicrobial resistance (%) among MRSA isolates (n=971), Hospital District of Southwest Finland, 2007–2016. Significantly increasing trends of resistance indicated with an asterisk in 2007–2010 (*) or 2011–2016 (**, Cochran-Armitage test for trend, $p\leq0.05$). Multi-resistant MRSA includes isolates with resistance to ≥2 of the tested antimicrobials. Adapted from Study II.

No significant changes were detected in the proportion of rifampicin, gentamicin or fusidic acid resistant isolates. Rifampicin and gentamicin resistance remained at a low and moderately low level (mean;range: 2.1%;0–4.3% for rifampicin and 4.7%;2–10.9% for gentamicin), respectively, throughout the study period. In 2009 and 2010, rifampicin resistance was not detected. Fusidic acid resistance remained at a moderate level (mean;range: 10.5%;7–14.8%) throughout the study period. No VRSA isolates were detected.

Regarding the multi-resistant MRSA phenotype, the proportion remained between 16.7% and 32% before 2011 without the presence of a clear trend. However, in the latter 2011–2016 period, an increasing trend ($p<0.001$) was observed resulting in a multi-resistance level of 39.2% of all MRSA isolates detected in 2016.

5.5 Multi-resistance across strain types, 2007–2016 (II)

The multi-resistant MRSA phenotype was detected in 248/971 (25.5%) isolates, representing 71 different *spa* types (**Table 2**, 3rd column). The most abundant *spa* types exhibiting a multi-resistant phenotype were t044 (23 isolates), t008 (22 isolates) and t034 (18 isolates). The most frequent resistance combination overall was clindamycin+erythromycin (*i.e.* an iMLSB resistance phenotype, see **Table 1**) detected in 126 isolates. However, within the most prevalent multi-resistant MRSA *spa* types, the most common phenotypes were fusidic acid+tetracycline for t044, erythromycin+levofloxacin for t008 and clindamycin+tetracycline for t034 isolates. Among all MRSA isolates, resistance to erythromycin (20.4%), tetracycline (16.3%) and clindamycin (15.8%) were the most frequent.

5.6 Risk factors of AMR, 2007–2016 (II)

For each antimicrobial individually and for multi-resistant MRSA, patient demographics were associated with resistant phenotypes (**Table 4**). Multi-resistant MRSA was associated (p<0.05) with cases defined as CA-MRSA, immigrant status, hospital care abroad and livestock contact, whereas no significant differences were observed for sex, household clusters and healthcare worker status. Non-multi-resistant MRSA was associated with older median age (48.5 vs 34.0 years, p<0.001), long-term care and cases defined as HA-MRSA. Clinical specimen types (vs screening specimens) were associated with multi-resistant MRSA.

For the individual antimicrobials with identified increasing resistance trends (clindamycin, erythromycin and tetracycline, **Figure 10**), the risk factors were similar to the multi-resistant MRSA (data found in Study II, Table S2). Clindamycin, erythromycin, tetracycline and fusidic acid resistant isolates were all associated individually with younger median age of patients than susceptible isolates. On the contrary, for levofloxacin, the median age of patients with resistant isolates was higher than those with susceptible isolates (58.3 vs 40.7 years, p=0.002). No significant differences were identified in the median age of patients between trimethoprim-sulfamethoxazole, gentamycin or rifampicin resistant and susceptible MRSA isolates.

Erythromycin, tetracycline and fusidic acid resistance were associated with cases defined as CA-MRSA isolates, whereas levofloxacin resistance was associated with cases defined as HA-MRSA. However, long term care was not identified as a risk factor of any additional resistance phenotype in MRSA isolates. Healthcare worker status was a risk factor for levofloxacin and trimethoprim-sulfamethoxazole resistance. Livestock contact was identified as a risk factor for clindamycin, tetracycline and trimethoprim-sulfamethoxazole resistance. Immigrant status was a

risk factor for clindamycin, erythromycin, tetracycline, and gentamycin resistance. Household clusters were associated with fusidic acid resistance. Hospital care abroad was a risk factor of resistance for all other antimicrobials than fusidic acid, and the only significant risk factor of rifampicin resistance in MRSA isolates. Erythromycin and levofloxacin resistance were significantly more abundant among clinical specimen types, than screening specimens.

Table 4. Risk factors associated with multi-resistant MRSA isolates, Hospital District of Southwest Finland, 2007–2016. Adapted from Study II.

	MULTI-RESISTANT MRSA[a] N (%)	MRSA N (%)	p^b
CASE COUNT	248	723	-
MALE SEX	119 (48.0)	321 (44.4)	0.337
MEDIAN AGE, IQR	34.0 (32.7)	48.5 (51.2)	**<0.001**
SCREENING SAMPLE	166 (66.9)	532 (73.6)	**0.049**
IMMIGRANT STATUS[c]	88 (35.5)	119 (16.5)	**<0.001**
HOSPITAL CARE ABROAD[d]	70 (28.2)	85 (11.8)	**<0.001**
LIVESTOCK CONTACT	20 (8.1)	2 (0.3)	**<0.001**
FAMILY CLUSTER	82 (33.1)	239 (33.1)	1.000
CA-MRSA*	94 (37.9)	211 (29.2)	**0.011**
HA-MRSA*	154 (62.1)	512 (70.8)	**0.011**
LONG-TERM CARE	11 (4.4)	161 (22.3)	**<0.001**
HEALTHCARE WORKER STATUS	22 (8.9)	47 (6.5)	0.251
HOSPITAL OUTBREAK	10 (4.0)	210 (29.0)	**<0.001**

[a]Resistance to ≥2 non-β-lactam antimicrobials, [b]Fisher's exact test or Mann-Whitney U test (Median age, interquartile range, IQR), significance level two-tailed p<0.05, significant values bolded. [c]Asylum seeker, refugee, resident of another country or other immigrant [d]Within two years, where information available. CA-/HA-MRSA* community-associated-/healthcare-associated methicillin-resistant *S. aureus* (see definition p. 47).

5.7 FTIR analysis of *S. aureus* isolates, 2023–2024 (III)

5.7.1 Isolate collection and *spa* types

A total of 178 staphylococcal isolates were collected in the Tyks Clinical microbiology laboratory during the collection period between September 2023–February 2024. As the isolates were collected in routine diagnostics, downstream WGS analysis revealed that one isolate (SWF_90) was indeed *Staphylococcus argenteus* (initially identified as *S. aureus* with MALDI-TOF) and was discarded

from the dataset, making the total number of isolates for further analysis 177. Of these, 88 were MRSA and 89 MSSA. MSSA isolates originated from blood culture specimens while MRSA isolates were derived from 54 screening specimens, 28 wound swabs, four blood cultures and two urine culture specimens.

All isolates were *spa* typed. A total of 86 different *spa* types were recorded. MRSA isolates were distributed to 41 and MSSA isolates into 58 different types. Thirteen *spa* types were detected among both MRSA and MSSA isolates (**Table 5**). As expected by the inclusion criteria, MSSA isolates represented a more diverse collection (58 vs 41 different *spa* types for MSSA and MRSA isolates, respectively). The most frequent *spa* types overall were t359 (10 isolates), t127 (10 isolates) and t008 (9 isolates), all predominantly MRSA isolates while among MSSA isolates, t267 (5 isolates) was the most frequent type.

5.7.2 FTIR clustering

All *S. aureus* isolates (n=177) were analysed with FTIR and one isolate (SWF_245) removed due to high technical variability despite repeated spectral acquisition (downstream isolate count of 176). Six spectra, including two biological replicates of three technical replicates, were included for each isolate, resulting in 1,056 individual spectra.

In the PCA model of all isolates, MRSA and MSSA isolates shared an overall high degree of phenotypic similarity (**Figure 10**, panel B). In this analysis, only two individual outlier isolates could be delineated (A blood culture MSSA isolate SWF_222/t172 and a screening MRSA isolate SWF_110/t172). Possible subclusters of two isolates could also be visually outlined (blood culture MSSA isolates SWF_171/t1575 and SWF_223/New type 2; screening MRSA isolates SWF_140/t011 and SWF_253/t2741).

To increase the resolution of the PCA clustering and to identify possible subtypes, the PCA model was applied to isolates sharing the same *spa* types. Within *spa* types, possible subtypes were visually outlined from a two-dimensional plot (data found in Study III, Figures S1–S16). With this approach, two possible phenotypic subtypes were detected among 10 different *spa* types, and three possible subtypes among five different *spa* types - and among singletons (**Table 5**). In the subtype analysis, six isolates of different *spa* types were considered nontypeable due to high technical variability between the spectra in the PCA plots.

MRSA and MSSA isolates were differentiated to distinct subtypes in 4 *spa* types (t359, t692, t021, t13909) and overlap of spectra from both MRSA and MSSA isolates was observed across 5 different *spa* types, while other *spa* types consisted of only MRSA/MSSA isolates or were singletons (**Table 5**). Among the 4 *spa* types where MRSA and MSSA isolates were separated between possible subtypes, the specimen

types were not different between the subtypes. Invasive MSSA isolates and mostly superficial MRSA isolates were hence clustered in the same possible subtypes.

Table 5. *spa* type distribution of MRSA and MSSA isolates (n=177) and the number of possible subtypes within *spa* types identified in the FTIR analysis, Tyks Clinical microbiology laboratory, September 2023 – February 2024. Adapted from Study III.

***SPA* TYPE(S)**	**TOTAL, N (%[a])**	**MRSA ISOLATES, N (%[b])**	**MSSA ISOLATES, N (%[b])**	**POSSIBLE SUBTYPES, N SUBTYPES (N IN EACH SUBTYPE)**	**DIFFERENTIATION OF MRSA AND MSSA ISOLATES BETWEEN POSSIBLE SUBTYPES**
t359	10 (5.6)	9 (90)	1 (10)	2 (9,1)	yes
t127	10 (5.6)	7 (70)	3 (30)	2 (2,8)	no
t008	9 (5.1)	6 (66.7)	3 (33.3)	3 (6,2,1)	no
t304	7 (4.0)	7 (100)	0 (0)	2 (5,1)†	only MRSA
t386	7 (4.0)	7 (100)	0 (0)	2 (1,6)	only MRSA
t172	7 (4.0)	3 (42.9)	4 (57.1)	2 (6,1)	no
t692	6 (3.4)	3 (50)	3 (50)	3 (1,2,3)	yes
t267	6 (3.4)	1 (16.7)	5 (83.3)	2 (4,1)†	no
t002	5 (2.8)	1 (20)	4 (80)	3 (1,2,2)	no
t355	4 (2.3)	4 (100)	0 (0)	1	only MRSA
t015	4 (2.3)	0 (0)	4 (100)	3 (1,1,1)†	only MSSA
t084	4 (2.3)	0 (0)	4 (100)	3 (1,1,2)	only MSSA
6 *SPA* TYPES WITH 3 ASSOCIATED ISOLATES EACH: t065, t091, t021, t024, t3841, t437	18 (10.2)	10 (55.6)	8 (44.4)	t021: 2 (1,1)† t437: 2 (1,1)† Others: 1	t021: yes t437: only MRSA Others: only MRSA/MSSA
12 *SPA* TYPES WITH 2 ASSOCIATED ISOLATES EACH: t1451, t362, t416, t521, t527, t005, t1309, t223, t693, t011, t034, t1597	24 (13.6)	10 (41.7)	14 (58.3)	t362: 2 (1,1) t1309: 2 (1,1) Others: 1	t362: only MSSA t13909: yes Others: only MRSA/MSSA
SINGLETONS*	56 (33.3)	21 (37.5)	36 (64.3)	3 (2,2,1)	-

[a]of all isolates, [b]within *spa* type *Includes New type 1, New type 2, New type 3, t026, t031, t046, t050, t073, t078, t105, t11450, t1146, t1231, t1362, t144, t148, t1575, t160, t1882, t189, t1994, t21155, t2119, t224, t228, t275, t330, t342, t4460, t5132, t5477, t571, t669, t715, t731, t872, t019, t10367, t1255, t15595, t20356, t21300, t216, t2174, t2330, t2741, t3220, t363, t4045, t4407, t441, t630, t665, t690, t726, t8290

†One isolate nontypeable due to high variation between biological/technical replicate

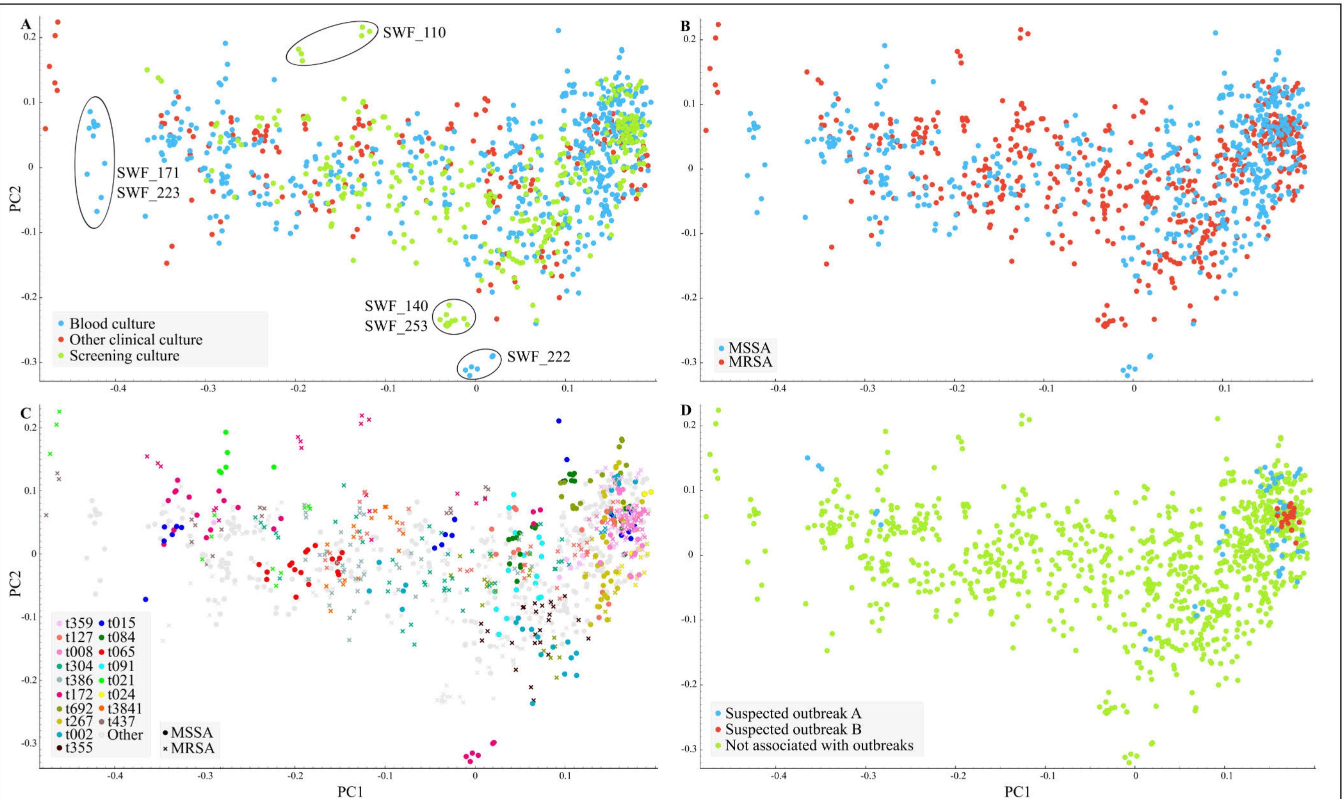

Figure 11. Two-dimensional scatter plot of the principal component analysis (PCA) model annotated by specimen type (**A**), methicillin susceptibility (**B**), *spa* type (**C**) and association to suspected outbreaks (**D**), *S. aureus* isolates (n=176), Tyks Clinical microbiology laboratory, 2023–2024. Each dot represents a spectrum, six replicate spectra per isolate. Wave number region 1,300–800 cm^{-1}. Phenotypic outlier isolates (SWF_110, SWF_222) and possible subclusters (SWF_140; SWF_253 and SWF_171; SWF_223) outlined. Visualised in Quasar (v.1.11.1) software[320]. Adapted from Study III.

5.7.3 Outbreak analysis

Two suspected outbreaks, A (SOA) and B (SOB), were detected during the collection period. The suspected outbreaks originated from the same healthcare facility, SOB following SOA with some six months in between the detection of the index cases. Initially, *spa* typing revealed type t359 as the primary strain among both outbreaks. To further assess the relationship between SOA and SOB, WGS and FTIR were applied to isolates from the suspected outbreaks. Partitions acquired with FTIR and *spa* typing were compared to that of WGS to evaluate the performance of the method in outbreak analysis. Due to small number of isolates in outbreak analysis, coefficients like Wallace and Rand were not appropriate to describe the relationship.

SOA comprised of nine isolates, of which five exhibited *spa* type t359 and four different *spa* types each (**Figure 12**). SOB comprised of four isolates, all t359. WGS analysis confirmed the close relatedness of t359 isolates within SOA (median pairwise distance 9 SNVs, range 4–13 SNVs) and the t359 isolates within SOB (median pairwise distance 10 SNVs, range 8–13 SNVs). The four non-t359 isolates of SOA were discovered unrelated to other isolates (pairwise distance >49 SNVs). A moderate degree of relatedness, suggesting shared evolutionary origin, was detected between the t359 isolates of SOA and SOB (median pairwise distance 39 SNVs, range 31–47 SNVs).

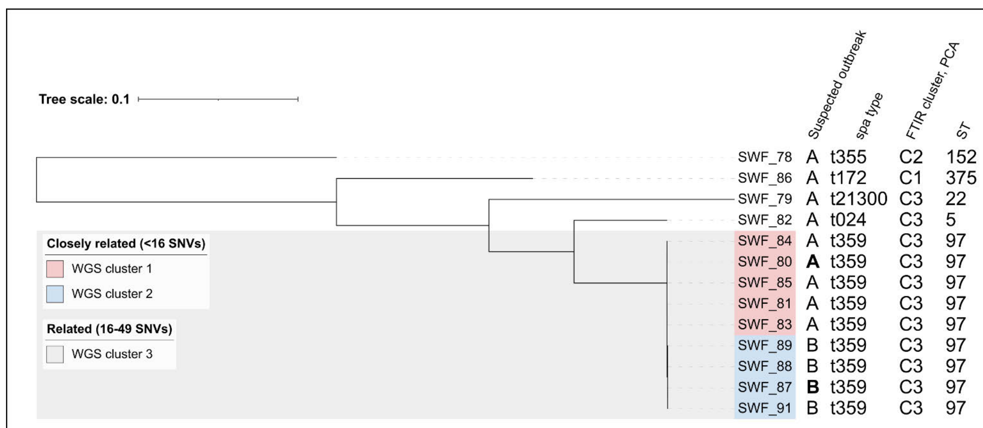

Figure 12. SNV-based dendrogram of the suspected outbreak isolates. Annotation: suspected outbreak (A or B), *spa* type, Fourier transform infrared spectroscopy cluster (FTIR cluster), and strain type. Related isolates in coloured ranges: closely related (red and blue), related (grey). Index cases bolded. Adapted from Study III.

In the FTIR-based PCA model, three clusters of spectra were observed (**Figure 13**).

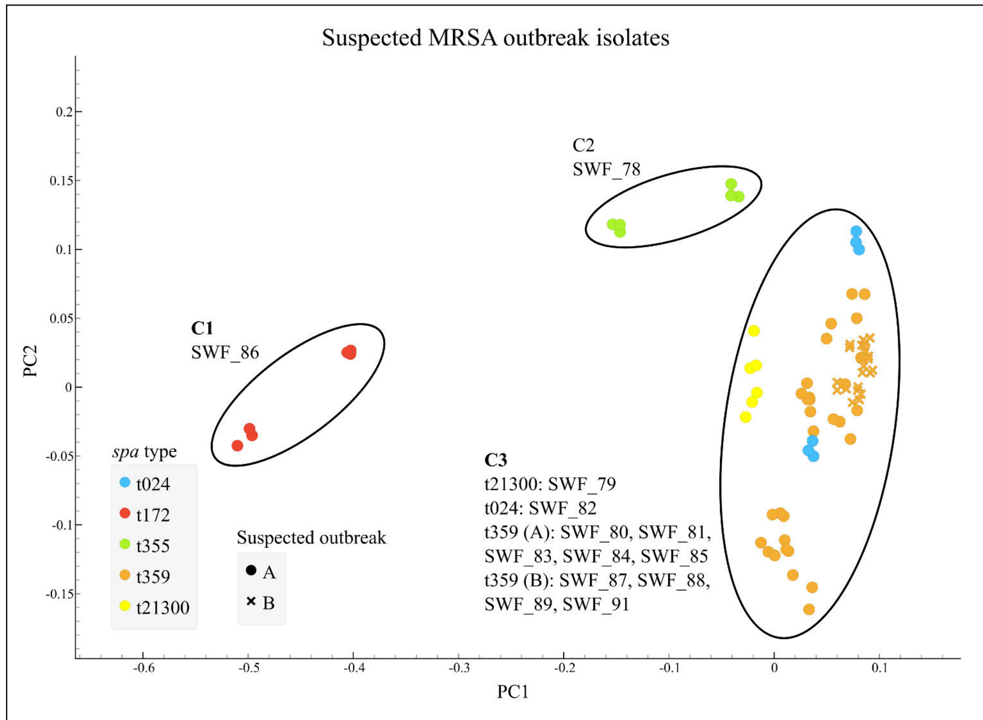

Figure 13. Two-dimensional scatter plot of the PCA model annotated by *spa* type and suspected outbreak association (n=13). Three possible clusters outlined (C1–C3), including outlier isolates SWF_86 (C1) and SWF_78 (C2) possibly separated from the cluster of other suspected outbreak isolates (C3). Overlap of spectra from both suspected outbreaks observed in C3. Adapted from Study III.

Two clusters (C1 and C2) consisted of spectra from a single isolate each and were outliers. Other isolates were clustered together in C3. The C3 cluster incorporated all t359 isolates from both outbreaks, and additionally a t024 and a t21300 isolate from SOA. As WGS analysis had demonstrated these isolates as unrelated, clustering of the non-t359 isolates was considered false positive typing results.

Because the clustering in the PCA model was subjective by nature, the HCA model was applied (**Figure 14**). Using isolate coherence (all spectra of a single isolate included within the same phylum) as a clustering threshold in HCA, the same result was obtained as with the PCA model. False positive clustering of two unrelated isolates of SOA (*spa* t21300-SWF_79) and (*spa* t024-SWF_82) was observed while two phenotypic outlier isolates (*spa* t172-SWF_86 and *spa* t355-SWF_78) were separated.

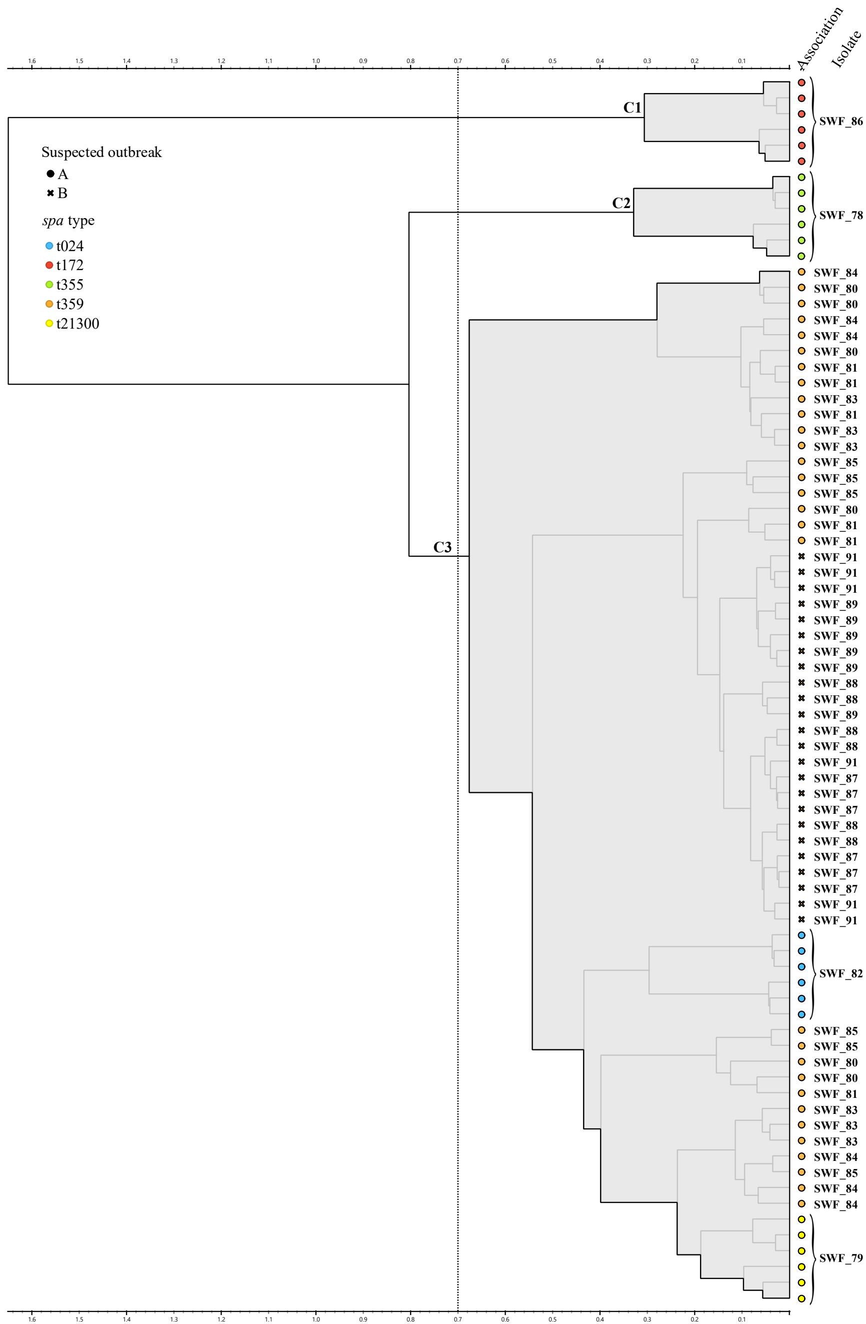

Figure 14. Hierarchical cluster analysis of the suspected outbreak isolates (n=13), 6 spectra per isolate. Euclidean distances and the Ward's linkage algorithm used. (C1–C3) cut-off was set to maximise cluster coherence (*i.e.* include all spectra of a single isolate). Adapted from Study III.

One possibility to reduce technical variation in the spectral analysis, is to apply an LDA, with the isolate name as the grouping identifier. Using PAST software, an LDA was performed, and the centroid axes used to infer an HCA with the Ward's linkage algorithm. Although with this approach no clustering threshold can be applied, the outbreak isolates were separated to different phyla than unrelated isolates, like in the WGS-based analysis (**Figure 15**).

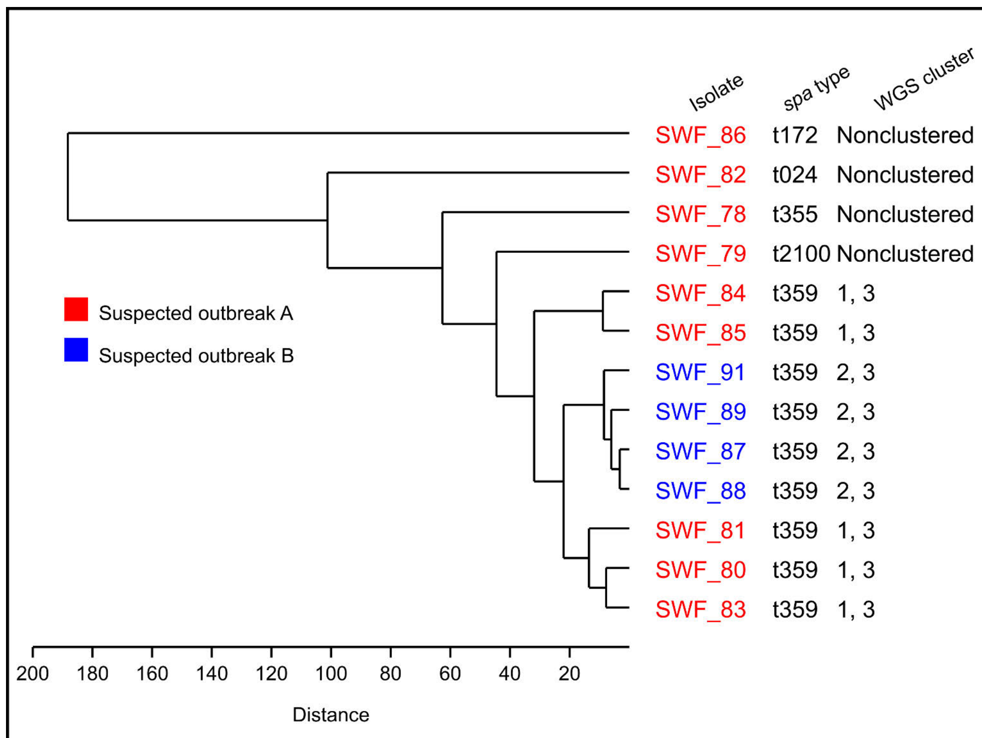

Figure 15. Linear discriminant analysis, centroid axes of each isolate used in Hierarchical cluster analysis with the Ward's linkage algorithm of the suspected outbreak isolates (n=13). *spa* type and WGS cluster (see Figure 11) of each isolate indicated. Author's figure.

6 Discussion

6.1 Changing epidemiology and strain types of MRSA in Southwest Finland, 2007–2016

During 2007–2016, a clear increase in the notification rate of MRSA (12.4 to 24.9/100,000 population annually) was observed in Southwest Finland[9]. The increase was characterized by epidemiological switch of MRSA acquisition towards younger age groups, especially young adults (decrease in median age from 48.5 to 39.1 years). Simultaneously, the HA-MRSA and CA-MRSA definitions we used continued to get more blurred, as lineages associated with community acquisition were increasingly detected from LTCFs and other healthcare institutions. In Studies I and II, we examined strain types associated with the changing MRSA epidemiology in Southwest Finland and observed increasing strain type diversity accompanied by the decline of a previously dominant t172 lineage.

These observations were concurrent with Norway, where increasing notification rate of MRSA was observed among both imported and domestic cases in people under 40 years of age (19.5 to 26.5% in 0–19-year-olds and 27.8 to 31.7% in 20–39-year-olds), 2006–2015[322]. These changes were accompanied by increases in foreign or unknown country of MRSA acquisition in Norway – and in Southwest Finland, the proportion of cases fulfilling the CA-MRSA definition and immigration-associated cases was increasing, making foreign acquisition and immigration as possible drivers of the increasing MRSA notification rate.

Immigration and MRSA acquisition abroad has been identified as drivers of the increasing MRSA notification rate in the Nordic countries during the 2010s[202]. In addition to aforementioned phenomena in Southwest Finland and Norway, in southern Sweden (Skåne), a year-by-year increase in both imported HA- and CA-MRSA cases was observed in 2000–2010, with the most prominent linkages of prevalent strain types to Middle-East (t044), the Balkans (t355) and Asia (t437)[221]. In Southwest Finland, the *spa* types t223 (spa-CC 790, **Table 2**) and t044 (spa-CC 4173/267) were the most common types associated with immigration[9].

In 2015, there was a notable increase in the number of refugees and asylum seekers in Europe, which also affected Finland. The proportion of population of foreign origin in Southwest Finland increased from 4% to 7% between 2007–

2016[190]. Among asylum seekers and refugees admitted to hospital, the prevalence of MRSA carriage (sampled from nose, pharynx, rectum, perineum, wounds) was 21% in the Helsinki and Uusimaa healthcare district, southern Finland, 2010–2017[222]. In the Netherlands, the positivity rate of screening specimens among asylum seekers was 10% for MRSA in 2015[323].

When looking at the strain types in Southwest Finland, a marked increase in the diversity of *spa* types was observed between 2007–2016[9]. In Study II, we examined the changing strain types further by applying clustering with the BURP method[277]. The clonal expansion and local replacement of predominant strain types is characteristic for MRSA[324].

With clustering, the year-by-year changes in the strain pattern could be clearly visualised: The spa-CC 172 predominated until 2009, after which it declined significantly. This was mostly caused by the decline of the prevalent founder *spa* type t172 in the cluster (237/273 of spa-CC 172 isolates). In addition to spa-CC 172, spa-CC 002 was declining. The spa-CC 002 is more diverse than spa-CC 172, with the clusters including 25 and 12 different *spa* types, respectively (**Table 2**). The most common *spa* types in spa-CC 002 were t002 (51 isolates), t688 (37 isolates) and t067 (15 isolates). The *spa* type t002 has been linked with the global HA-MRSA CC5 lineage, which has been present in Finland already in the 1990s, when it caused large healthcare outbreaks[189]. It has also been reported from Sweden in the 2010s[221]. In 2018, it was reported from Poland, colonizing patients with cystic fibrosis, a patient group with frequent healthcare contact[325]. *spa* type t067 was responsible for the large outbreak of Pirkanmaa area, 2001–2011, and detections in Southwest Finland during 2007–2016 may reflect transmission from the neighbouring district, but confirmation of this spillover is lacking[326]. Before the epidemic of Pirkanmaa, t067 had been reported from multiple HAOs in Spain[327]. *spa* t688 has been associated with healthcare transmission, and according to the Ridom spaserver, detections occurred in many European countries during 2007–2016 (Norway, Iceland, Switzerland, Germany) and more recently in the 2020s, multiple detections have been reported from Germany[9,272,328].

We observed a peak in spa-CC 008 isolates in 2007, which was explained by an outbreak in a healthcare institution[9]. During years following 2007, the proportion of spa-CC 008 isolates again slowly increased (6.2%–16.2%, 2008–2016). The most abundant *spa* types in spa-CC 008 were t008 (73 isolates) and t304 (22 isolates, **Table 2**). The t008 has been associated with the of successful CA-MRSA USA300 clone, and has been established in the country since the emergence of CA-MRSA in Finland[156,201,210]. It was the second most abundant *spa* type in Southwest Finland, 2007–2016[9]. Although we observed a decline in the proportion of t008 in Southwest Finland, it remained as the most prevalent strain type in the whole country in 2023, with 16% of all MRSA isolates exhibiting t008[8]. Additionally, the t008 isolates were

the most abundant in people over 75 years of age, and they were associated with PVL positivity[8]. t008 strains have been recently associated with invasive infections of intravenous drug users in Finland[10]. Similar observations were made for t304: it was the second most abundant *spa* type isolated overall (13% of all MRSA isolates), and from people over 75 years of age in 2023, and caused invasive infections among intravenous drug users in the Pirkanmaa area[8,10]. For t304, a link with immigration from the Middle-East has been suggested, and increase in its detection was reported between 2009–2016 from Sweden, Norway and Denmark[202,222].

The spa-CC 790 was the third most prevalent cluster overall, representing 25 different *spa* types and 122 (12.6% of all) isolates (**Table 2**). Its proportion saw changes during 2007–2016, peaking in 2010, when it represented 28.6% of all isolates, declining to 5.2% in 2012, followed by an increase to 19.5% in 2016. The most prevalent *spa* types in spa-CC 790 were t032 (40 isolates) and t223 (38 isolates). The *spa* t032 has been identified as a high-risk HA-MRSA clone associated with clinical infections in Southwest Finland and invasive infections in Pirkanmaa[9,225]. It represents the HA-MRSA lineage with ST22, which has been documented to effectively transmit in the healthcare environment and caused a large hospital outbreak in Denmark in the 2000s[329]. It has a widespread occurrence, including the UK (EMRSA-15), Australia and China[163,330,331]. The ST22 isolates exhibited increased virulence in comparison to the USA300 and ST59 isolates in *Galleria mellonella* infection and mouse skin abscess models[331]. The t223 isolates, like t304, were increasingly detected in Sweden, Norway and Denmark in 2009–2016 and in Norway, linkages to immigration from Syria, Russia and Afghanistan were identified[202,322]. Interestingly in contrast to the HA-MRSA spa-CC 790 clone t032, t223 was associated with community acquisition and immigration in Southwest Finland[9]. This reflects the success and transmission potential of these spa-CC 790 strains in both healthcare and community settings. However, with the lack of genomic comparison, the degree of relatedness between t223 and t032 isolates remains unclear.

In Study II, we observed an increasing proportion of isolates in the spa-CCs 012 (from 3.6% to 17.7%) and 4173/267 (from 1.8% to 9.7%), 2007–2016, in addition to small spa-CCs and singleton *spa* types. Although smaller than previously described clusters, spa-CC 012 (81 isolates) and 4173/267 (58 isolates) were diverse, representing 20 and 13 different *spa* types, respectively (**Table 2**). The most common *spa* types in spa-CC 012 were t019 (24 isolates) and t034 (20 isolates), while for spa-CC 4173/267 the most prevalent *spa* type was t044 (34 isolates). The *spa* t019 is a PVL-positive CA-MRSA clone, associated with the ST30 lineage, and has been described among healthcare outbreaks in several Danish hospitals since 2005[9,332]. In Sweden, PVL-positive t019 together with t008 and t044 strains were emerging during 2000–2010 and caused significantly more SSTIs than other

types[221]. Recently in 2019–2022, SCC*mec* IV carrying CC30/t019 clone was described as a major genotype causing external otitis in Iran[333]. The t044 *spa* type is also a CA-MRSA lineage, usually PVL-positive, and was frequently detected among people with a temporary personal identification number (recently arrived) in Finland in 2015[334]. It was also emerging in Sweden, 2000–2010, where it was linked with immigration from the Middle East[221]. The t044 *spa* type was the most prevalent type among hospital MRSA isolates from Jordan, 2009–2010[335].

Lastly, the *spa* t034, associated with the CC398, has been the most common LA-MRSA type observed in Southwest Finland and nationally[8,9]. The MRSA-t034 cases in Southwest Finland mostly represent contacts with pigs, and the proportion of CC398 isolates has remained <10% of all annual isolates[8,9]. LA-MRSA does not appear to be widely spread in Finland in comparison to some other European countries with industrial pig production, such as Denmark and the Netherlands, where the proportion of CC398 isolates of all human MRSA detection was >10% with more than 100 reported cases in 2013[336]. In 2024, a total of 744 CC398 MRSA cases were notified in Denmark, corresponding to 22% of all new MRSA cases[337]. The number of CC398 MRSA identifications have been rising also in Finland; from 10 to 50 cases per year, 2014–2024[4]. LA-MRSA appears to be adapted for human-to-human transmission in the community, including to risk populations, as many cases are reported without known exposure and 16% of all invasive MRSA infections in Denmark being CC398 in 2014 and 2024[224,337].

6.2 Changes in antimicrobial resistance of MRSA, Southwest Finland, 2007–2016

Awareness in the changes of MRSA antimicrobial susceptibility pattern is vital for empiric treatment guidelines in a low-endemicity setting[93]. To date, non-susceptibility of MRSA to last resort antimicrobials, glycopeptides, in Finland has not been detected.

In Study II, we observed a notable increase in the proportion of multi-resistant MRSA (resistance detected to ≥2 of the tested non-β-lactam antimicrobials). The proportion of multi-resistant MRSA increased from 15% to almost 40%, 2007–2016. Accounting for the change in the interpretation of AST results from the CLSI standard to EUCAST in 2011, trend analysis was conducted independently for time periods 2007–2010 and 2011–2016. While in the first period, no significant increasing trend was detected, the second period showed a statistically significant increase in the proportion of multi-resistant MRSA, which coincided with the increases in number of strain types included in spa-CCs 012 and 4173/267. The increase of multi-resistance was mainly caused by changing resistance rates to

clindamycin, tetracycline and erythromycin, which were all increasing in the latter 2011–2016 period.

Similar findings of increasing AMR among MRSA isolates were reported concurrently from a large study including 148,561 isolates from Germany 2010–2015, where they discovered an overall decrease in the proportion of MRSA isolates across all sample types (16% to 10%), but an increasing resistance of MRSA isolates to tetracycline and gentamycin[338]. Similarly to Southwest Finland, tetracycline resistance in MRSA was associated with outpatient settings and younger age groups, possibly suggesting a link to LA-MRSA, where tetracycline resistance is characteristic[338,339]. Overall, tetracycline resistance of MRSA in Southwest Finland has remained at a heightened level (>30%) in comparison to *e.g.* Denmark, where the non-LA-MRSA tetracycline resistance has remained between 21–24%, 2021–2024 and Norway, where tetracycline resistance did not increase above 30% of all tested MRSA isolates until 2023[337,340,341].

Recent analysis from the USA reported a decrease in the proportion of MRSA in a nationwide collection of outpatient isolates (from 53.6 to 38.8% between 2010 and 2019), while among the MRSA isolates, tetracycline, trimethoprim-sulfamethoxazole, and clindamycin resistance were increasing[342]. In Finland, the most recent report on the national surveillance of AMR (FinRES), the proportion of MRSA among all tested *S. aureus* isolates was slightly increasing (from 2.4 to 3.1% between 2022–2023), and while resistance rates to clindamycin and erythromycin showed small increases, the proportion of co-resistant strains among all tested *S. aureus* strains remained low (erythromycin+MRSA 1.5%, clindamycin+MRSA 1.1%)[343].

6.3 Prospects for MRSA control in Southwest Finland

Public reports of the Tyks Clinical microbiology laboratory indicate that the proportion of AMR among MRSA isolates has remained at a similar, or higher, level as reported in Study II, for clindamycin, erythromycin and tetracycline – whereas levofloxacin resistance has again shown year-by-year increases, reaching 23% in 2023[340]. Overall, AMR to many non-β-lactams in MRSA isolates appears to be higher than MSSA isolates in Southwest Finland (2023: erythromycin 7% vs 37 %, clindamycin 8% vs 30%, gentamycin 0.7% vs 9%, levofloxacin 2% vs 23 % for MSSA and MRSA, respectively)[340]. This observation suggests that the changes reported in AMR profile of MRSA isolates in Study II are long-lasting and may provide additional challenges for the management of non-invasive MRSA infections in Southwest Finland in the future.

By the end of 2025, the notification rate of MRSA in Southwest Finland has continued to increase (34.6 to 40.8 per 100,000 population between 2024–2025), and worryingly, the incidence of MRSA BSI is increasing rapidly (0.6–3.0 per 100,000 population between 2024–2025) warranting rapid control measures[4]. This phenomenon contrasts with the relatively large proportion of screening specimens among all MRSA cases in 2007–2016 (**Figure 7**).

Epidemiological analysis presented in this thesis were congruent with the current inclusion of specific epidemiological risk groups to the local screening schema upon hospital admission: hospital care abroad within one year, contacts to livestock (pig production), history of intravenous drug use and household contact to a known MRSA carrier[344]. The previously reported critical role of households as a possible driver of MRSA to endemicity, was also reflected in our results: t172 strains appeared to persist in multiple FCs[345]. The substantial risk of MRSA transmission (among other MDR pathogens) in direct patient transfers from hospitals of conflict areas has also been recognized[230,231]. However, as the proportion of community-acquisition of MRSA was increasing in our data, 2007–2016, accompanied by the presence of multi-resistant MRSA strains in the community, further analysis on the current epidemiology and possible strategies to enhance MRSA detections and surveillance in the community (*e.g.* via primary healthcare practitioners, needle exchange programmes[10]) could be explored.

In the national guideline for the prevention of MDR pathogens, *spa* typing is implemented as an initial tool to assess whether MRSA cases in the same healthcare facility are linked (outbreak signal warranting further investigation)[346]. However, for regionally common *spa* types, the resolution of *spa* typing could be questioned in this indication. As shown in Studies I and III, local MRSA detections of common *spa* types can be clonal (confirm transmission) or differ significantly (refuting transmission). In these situations, outbreaks could be more precisely analysed with a stronger implementation of a local targeted WGS typing strategy for MRSA. Rapid methodology such as the FTIR could help to curb costs currently associated with WGS.

6.4 Analysis of the t172 MRSA strains

The decline of the dominant t172 MRSA lineage in Southwest Finland was apparent in 2007–2016. Transmission within households has been suggested as an important way for MRSA to sustain and expand in the community, and the t172 isolates were predominant in MRSA family clusters in Southwest Finland, 2007–2016[9,347,348]. Using WGS in Study I, we were able to observe moderate genetic variation within the t172 clone. We identified multiple transmission clusters of t172 subclones. By looking at index cases from healthcare outbreaks (HAOs) and family clusters (FCs),

we observed 8 previously undetected transmission clusters. Seven out of the eight clusters would not have been detected without the WGS analysis due to the lack of an epidemiological link or temporal distance.

The biggest cluster 1 included fourteen isolates, and showed a link between four different FCs, three HAOs and four sporadic cases (which were defined as isolates without an epidemiological link to a FC or HAO). Although the direction of transmission could not be inferred from the WGS analysis, the detection time of isolates in cluster 1 spanned 2009–2013. The prolonged detection of a single t172 clone highlights the effective transmission in different settings (healthcare and the community) and persistence of this lineage in Southwest Finland. This suggests a long-term local reservoir of t172 in Southwest Finland, where the household transmission likely plays an important role. Adaptation of a targeted sequencing schema for identified high-risk clones (such as sporadic t172 isolates), could be used as an alternative to routine WGS to identify possible transmission routes and target preventative measures (screening, decolonisation, contact precautions in healthcare) to limit the spread of MRSA[348].

In Study I, we also characterized the demographic features associated with the *spa* type t172 strains. It was recognized a primarily Finnish strain type, as it is rarely reported elsewhere in the world, including the Nordics[202]. In Finland however, it has consistently remained as one of the most prevalent strain types until recent years, especially in people with ≥75 years of age[8,202]. A related *spa* type t437 of the CC59 lineage, has been identified across Europe and possibly imported multiple times from China, where the CC59 is a predominant CA-MRSA lineage[59,215].

The decline in the proportion of t172 in Southwest Finland coincided with the increase in new strain types of spa-CCs 012 and 4173/267. The spatiotemporal clonal replacement of strain types is a well-recorded feature in MRSA epidemiology[213,217,349]. Contradicting the emerging strain types, the t172 strain was strongly associated with an older patient population, local household and healthcare-associated transmission clusters and narrow spectrum of AMR to non-β-lactams. While the t172 isolates were in decline in our study, the demographic features associated with the clone suggest that clonal replacement with the emerging clones is not solely behind the decline – and may reflect successes in controlling the transmission of MRSA-t172 in healthcare institutions of Southwest Finland. On the other hand, the emerging clones were associated with different ecological niches (younger median age, immigration, travel and work abroad and hospital care abroad, **Table 3**) to t172. We did, however, continue to sporadically detect t172 MRSA (and MSSA) isolates in Southwest Finland in Study III (**Table 5**).

6.5 Prospects for strain typing of *S. aureus* with FTIR

While WGS shows excellent resolution in staphylococcal typing and can effectively complement routine *spa* typing in outbreak analysis, a quicker and cheap typing method could be helpful for timely and cost-efficient infection control. In a prospective setting in Study III, we evaluated the performance of a rapid typing method, FTIR, for strain typing of MSSA and MRSA isolates and applied the method for outbreak investigation. With 177 *S. aureus* isolates collected from Southwest Finland, 2023–2024, we detected an overall high phenotypic similarity between MRSA and MSSA isolates. For most *spa* types, the separation of MRSA and MSSA was not possible. This likely results from the intrinsic feature of the FTIR method, since the clustering is based on a spectral window corresponding to polysaccharide structures, which define serotypes (800–1,300 cm^{-1}), rather than the defining structure of the MRSA phenotype (peptides, PBPs)[238].

Within 16 *spa* types, we were able to detect phenotypic subtypes, reflecting the different resolution of FTIR and *spa* typing[350]. The subtypes within *spa* types rarely differed by methicillin susceptibility or specimen type, and without further WGS, the basis for the differences between the possible subtypes remained inexplicable.

In the outbreak analysis with FTIR, we achieved good concordance with WGS based analysis. The outbreak analysis consisted of 14 isolates with typing results including *spa* typing, WGS and FTIR. With FTIR, using the unsupervised chemometric analysis PCA, we observed false positive clustering of two isolates that were considered unrelated to the outbreaks with both WGS and *spa* typing. However, when applying supervised chemometrics (linear discriminant analysis, LDA), the result was comparable to WGS and *spa* typing. Although a clear clustering threshold in LDA could not be applied, the topology of the tree supported partition similar to that of WGS and *spa* typing. No false negative clustering was observed in either PCA or LDA, which has been the case also in previous studies reporting on adequate negative predictive value of FTIR for *S. aureus,* in clinical settings and food isolates[248,308,310].

The FTIR method is highly sensitive to changes in the laboratory protocol, including the phase of bacterial growth, incubation atmosphere, temperature and culture medium[238,306]. The effects of the technical variation can be mitigated by including technical and biological replicates to the analysis. Additionally, by applying supervised chemometrics such as LDA, technical variation between spectra can be reduced[238,321].

The sensitivity of the method to small changes introduced likely by culture conditions or laboratory workflow was the main challenge we faced when applying the method. Also, the complexity of spectral data analysis was an issue, as the performance of the primary FTIR analysis software was reduced when the number of isolates exceeded ca. 20 isolates. Inclusion of technical and biological replicates

also complicated the analysis, as 20 isolates already corresponded to 120 individual spectra (minimum of 3 technical and 2 biological replicates).

In conclusion, our results support a suggested role of FTIR spectroscopy as a preliminary, rapid screening tool for suspected outbreak isolates, with a possibility to reduce WGS analysis of prospective unrelated isolates[310]. The advantages of FTIR include speed, high throughput and low cost of analysis per specimen in comparison with WGS[305]. It could be an attractive method for prolonged or large outbreak analysis, where access or resources to perform WGS are limited.

6.6 Strengths and limitations

In studies I and II, a large population-based material consisting of detailed epidemiological risk factors was available, which enabled a comprehensive and detailed analysis of demographic risk factors, strain types and MRSA resistance phenotypes. The material covered a ten-year period which allowed for the reliable analysis of trends, rather than just sporadic events. The mandatory, laboratory-based surveillance and reporting of MRSA cases to healthcare registries (NIDR and HILMO at THL) and hospital records, including the regional register of hospital infections and carriers of resistant bacteria (SAI register) reduced the risk of selection bias, when looking at the complete regional data, rather than a single-centre. On the other hand, including complete national surveillance data could have diluted the regional effects, potentially masking the detailed epidemiological phenomena specific to the WSC of Southwest Finland.

The uniform typing, and AST data were possible to be acquired largely due to the strain banks collected in the Tyks Clinical Microbiology Laboratory and THL, where all new MRSA isolates are stored. As *spa* typing is performed in THL for all new MRSA isolates, in-house *spa* typing was required only for isolates preceding the national routine adaptation of *spa* typing in 2009 and for the prospectively collected MSSA isolates[213]. Cooperation with the THL and Tyks laboratories enabled the verification of *spa* typing results.

Limitations of the study include geographic limitation to one healthcare district or wellbeing services county in Finland, which limits the generalisability of our results. However, in low-endemicity settings, similar phenomena can drive the emergence of new MRSA strain types, such as cross-country influx with the movement of people and animals[351]. A possible limitation is the time period included in the studies, 2007–2016, which reflect the situation at 10 years ago in 2026. Risk factors and circulating strain types may have significantly changed during the last 10 years, which would be important to analyse in the future. Nevertheless, the changed pattern of AMR of MRSA isolates in Southwest Finland has remained after 2016 until recently, as discussed above.

Information on the hospital care abroad during the last two years was collected for all cases throughout 2007–2014, but for some cases in 2015–2016 hospital care abroad possibly included only the last one year (see chapter 4.2). Because collected information uniformly included hospital care abroad during the previous one year, we could assign these cases to HA-MRSA. This resulted to some cases, with a recorded hospital care abroad during the last 1–2 years, to be included in the CA-MRSA group in studies I and II - as they did not fulfil the chosen HA-MRSA definition. We identified 24 CA-MRSA cases with hospital care abroad within the last 1–2 years. None of these cases represented *spa* type t172, so the impact on the characterisation of t172 as a primarily Finnish CA-MRSA strain type would not be impacted by this. In Study II, hospital care abroad within 2 years was identified as an independent risk factor of multi-resistant MRSA, and to all studied antimicrobials (other than fusidic acid) individually. However, association of multi-resistant MRSA phenotype and resistance to some antimicrobial agents individually with CA-MRSA may have been affected by our decision in defining HA-MRSA (including one year of previous hospital care abroad) as some cases assigned to CA-MRSA from 2015–2016 may represent cases that had been in hospital care abroad within 1–2 years.

An important limitation for Studies I and III is the lack of WGS confirmation for all isolates included in the studies, which limits the power of our conclusions when comparing different strain typing methods. To overcome the limited availability of WGS typing, we applied careful selection of isolates for WGS in both studies I and III to obtain desired information on the transmission of t172 isolates in Study I, and outbreaks analysis in Study III. Additionally, a comprehensive strain collection enabled us to pursue this selective sequencing strategy. In one hand, this strategy is more resource-efficient than universal sequencing and could be further applied in the future to support local infection control. On the other hand, increased sequencing capacity in regional clinical microbiology laboratories could also be used to strengthen national MRSA surveillance in Finland.

One important consideration when applying a WGS protocol, is the definition of clustering cutoff. As seen in Study I, some isolates were 1–3 SNVs away from the clustering threshold, so the choice of a clustering threshold can have significant impact on the observed clustering. For *S. aureus,* strains with strong epidemiological links usually tend to differ from each other by under 20 core-genome SNVs[316,352]. We decided to use a genetic relatedness cutoff suggested by Coll *et al.* in 2020, where they analysed over 1,000 MRSA isolates and established 15 core-genome SNVs as a threshold to rule out transmission events within 6 months[314]. As in Study I, we observed clustering in isolates with isolation dates spanning >1 year using the 15 SNVs threshold, a less stringent threshold could have well been used to more clearly separate the nonclustered isolates. Nonetheless, a stringent threshold increases the credibility of the observed clusters.

7 Summary & Conclusions

The main findings and conclusions of this thesis included the following:

1. The increased notification rate of MRSA in Southwest Finland, 2007–2016, was characterized by an increasing strain type diversity, as defined by *spa* typing and spa-CC clusters.
2. The previously dominant MRSA strain, *spa* t172, was declining in proportion in 2007–2016 and strong indications of primarily domestic transmission of this strain type was observed.
3. WGS revealed persistent circulation of multiple *spa* t172 MRSA clones in healthcare institutions and households of Southwest Finland during 2007–2016. New, previously undetected links between the t172 subclusters spanned multiple years and confirmed MRSA transmission between healthcare and community settings.
4. The increased notification rate of MRSA in Southwest Finland, 2007–2016, was accompanied by a switch in the AMR profile of MRSA isolates towards increased multi-drug resistance. Specifically, increasing resistance trends were detected for tetracycline, erythromycin and clindamycin, and increased AMR was highlighted in younger age groups. Increased AMR in cases defined as CA-MRSA isolates was observed.
5. WGS, *spa* typing and FTIR methods together offer powerful tools to assess *S. aureus* strain relatedness in a low-endemicity setting.
6. FTIR shows promise as an emerging rapid tool for *S. aureus* outbreak identification. A previously suggested possibility to reliably refute identified MRSA cases from outbreaks with FTIR was supported in our results. This strategy could reduce the need for more expensive WGS analysis.

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