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MOLECULAR DIAGNOSTICS IN MICROBIOLOGY

# Contemporary genomic approaches in the diagnosis and typing of *Staphylococcus aureus*

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**Summary**

*Staphylococcus aureus* is a major human pathogen, causing disease in both community and healthcare settings. Over the past two decades, the epidemiology of *S. aureus* disease has changed dramatically, with the emergence and spread of community-associated methicillin-resistant *S. aureus* clones. This epidemiological shift, coupled with the association between delayed antimicrobial therapy and increased mortality in *S. aureus* bacteraemia, has greatly facilitated advances in the rapid molecular diagnosis of *S. aureus.* Rapid molecular testing for *S. aureus* can greatly reduce laboratory turnaround time, and in some circumstances, may lead to improved clinical outcomes. In addition, advances in DNA sequencing technology and bioinformatic analysis have shed new lights on the molecular epidemiology and transmission dynamics of *S. aureus.* In this context, we provide an overview of the key advances in the molecular diagnosis and typing of *S. aureus*, with a particular focus on the clinical impact and utility of genomic technologies.

*Key words:* Molecular, MRSA, PCR, sequencing, *Staphylococcus aureus*.

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## INTRODUCTION

*Staphylococcus aureus* is a major human pathogen, causing considerable morbidity and mortality globally.[1] Infections caused by *S. aureus* range from non-invasive disease such as skin and soft tissue infection, to severe conditions such as osteomyelitis, endocarditis and sepsis.[2] In addition, *S. aureus* is a frequent coloniser, found in approximately 20–30% of people without causing clinical disease.[3] A number of factors contribute to the success of *S. aureus* as both a commensal and a pathogen. These include an array of virulence determinants, as well as the capacity to successfully acquire numerous antimicrobial resistance determinants.[4–6]

The first methicillin-resistant *S. aureus* (MRSA) strains were reported in the early 1960s,[7] only a few months after the introduction of this antimicrobial for human use. In the 1980s and early 1990s, MRSA emerged as a major problem in healthcare facilities, largely due to the spread of epidemic clones of healthcare-associated MRSA (HA-MRSA).[8] However, since the mid-late 1990s, the clinical and molecular epidemiology of *S. aureus* disease has changed considerably, both in Australasia and beyond.[1,9] In particular, the emergence of community-associated MRSA (CA-MRSA) infections in young patients with no preceding healthcare contact, and the epidemic spread of CA-MRSA clones have changed the landscape of *S. aureus* disease in the 21$^{st}$ century.[10] Moreover, advances in molecular techniques, particularly in molecular typing methods, have led to novel insights into the transmission and spread of *S. aureus* in both community and healthcare settings.

In this context, the purpose of this review is to provide an overview of key advances in the molecular diagnosis and typing of *S. aureus*, with a particular focus on the clinical impact and utility of genomic technologies.

## RAPID MOLECULAR DETECTION OF *S. AUREUS* FROM CLINICAL SPECIMENS

At present, the cornerstone of microbiological diagnosis of *S. aureus* infection remains the growth of *S. aureus* from clinical specimens. However, there are notable limitations with standard phenotypic methods, particularly relating to turnaround time for conventional culture and antimicrobial susceptibility testing (AST). Over the past decade, a number of rapid molecular tests have been developed. These are generally based on identification of a *S. aureus* species-specific marker, with or without detection of *mecA*, the gene responsible for production of the atypical penicillin-binding protein (PBP2a) that confers methicillin resistance in staphylococcal species.[11–13] To date, the two most common clinical situations where these tests have been employed are: (i) in the rapid speciation and *mecA* profiling of *S. aureus* from blood cultures, and (ii) screening for the presence of *S. aureus*, particularly MRSA, in the context of infection prevention and control.

### Rapid detection of *S. aureus* from blood cultures

*Staphylococcus aureus* is one of the most common causes of bloodstream infections, in both hospital and community settings.[14] Despite advances in modern healthcare, *S. aureus* bacteraemia is still associated with considerable mortality, with one prospective Australasian study from 2009 describing a 30-day all-cause mortality rate of approximately 20%.[15] Several studies have highlighted the detrimental effects of inappropriate empiric or delayed antimicrobial therapy on clinical outcomes of *S. aureus* bacteraemia.[16] For example, a recent meta-analysis assessed 510 episodes of MRSA bloodstream infection, and found that the 30-day all-cause mortality was significantly higher in patients receiving inappropriate empiric therapy compared to patients receiving appropriate treatment (49.1% versus 33.3%; $p < 0.001$).[17] Similarly, a

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study of hospital-onset *S. aureus* bacteraemia found that delayed antimicrobial treatment was an independent predictor of *S. aureus* bacteraemia-related death [odds ratio (OR) 3.8; 95% confidence interval (CI) 1.3–11.0], with MRSA infection the most significant factor associated with delayed appropriate therapy (OR 8.3; 95% CI 2.6–16.8).[18] In theory, rapid differentiation of *S. aureus* from other staphylococcal species, along with antimicrobial resistance profiling, should allow early and definitive antimicrobial treatment to be instituted.

In the past, conventional diagnosis of *S. aureus* bacteraemia involved growth of bacteria in blood culture media, followed by presumptive identification of Gram positive cocci (GPC) in clusters, and then plating onto solid media for identification and susceptibility testing. Depending on workflow within individual laboratories, this process could take between 48 and 72 hours. In some laboratories, the use of chromogenic agar had decreased the turnaround time for rapid speciation and detection of MRSA, although this approach still required prolonged incubation.[19,20] The advent of matrix-assisted laser desorption ionisation–time of flight (MALD-TOF) mass spectrometry in clinical laboratories has greatly reduced the time to definitive identification of bacteria from screening swabs or from critical clinical specimens including blood cultures. Methods such as pellet purification, lysis centrifugation and rapid subculture followed by MALDI-TOF mass spectrometry can provide final identification in 1–6 hours from the time of blood culture growth detection.[21–23] Any rapid molecular methods must now be considered with this improved benchmark in mind.

A number of molecular platforms have been developed to facilitate the rapid diagnosis of *S. aureus* bacteraemia; these tests generally involve detection of *S. aureus* from blood culture bottles, or less commonly, detection of *S. aureus* directly from blood taken from a patient with clinical signs of sepsis.[11,13]

The most commonly utilised molecular tests in the rapid diagnosis of *S. aureus* bacteraemia are those employed when a blood culture bottle is found to contain GPC resembling staphylococci.[12] An aliquot of the blood culture broth is used for subsequent molecular testing, generally using PCR amplification, followed by a number of detection methods (e.g., fluorescent probes for real-time detection, or microarray hybridisation). PCR-based assays, such as the Cepheid Xpert MRSA/SA Blood Culture assay[24] and the BD GeneOhm StaphSR assay,[25,26] are commonly used molecular tests for direct detection of *S. aureus* from blood cultures. When compared to conventional culture methods, both assays are reported to have high sensitivity and specificity for the identification of methicillin-susceptible *S. aureus* (MSSA) and MRSA, with a manufacturer's report suggesting 98.8–100% sensitivity and 97.2–100% specificity for the BD GeneOhm StaphSR assay,[12] and similar high reported rates of sensitivity and specificity for the Cepheid Xpert MRSA/SA assay.[24,27,28]

In order to differentiate between *S. aureus* and other staphylococcal species, primers for a *S. aureus* species-specific marker are generally incorporated into molecular assays. The most common *S. aureus* species markers used to date are the staphylococcal nuclease (*nuc*) gene or the staphylococcal protein A (*spa*) gene. Rapid detection of methicillin resistance is performed by detection of the *mecA* gene, although there is potential for false detection of MRSA in mixed specimens containing *mecA*-harbouring methicillin-resistant coagulase-negative staphylococci (CoNS) and MSSA.[24,29] In order to overcome this, most molecular assays also contain primers targeting the region between the *mecA*-containing staphylococcal chromosomal cassette (SCC*mec*) and the *orfX* gene, a gene unique to *S. aureus*, located where the SCC*mec* element inserts into the *S. aureus* chromosome. In some *S. aureus* strains, there may be an SCC element that lacks the *mecA* gene, so called 'empty cassette variants'.[24] For example, MSSA476 contains a 23 kb SCC element ($SCC_{476}$) that carries a fusidic acid resistance determinant, but does not contain *mecA*.[30] In assays that only target a *S. aureus* species marker and the *orfX*-SCC*mec* junction, these 'empty cassette variants' may be reported as MRSA, although phenotypically and genotypically they are actually methicillin susceptible.[25] Such false-positive results may lead to inappropriate antimicrobial treatment, and/or unnecessary isolation of patients. Moreover, one Australian study highlighted the potential for false-negative MRSA results due to genetic variation in the junctional *orfX*-SCC*mec* target of the SCC*mec* region.[31]

In addition to PCR-based molecular assays, the Verigene Gram positive blood culture (BC-GP) test is an automated microarray-based platform that can identify several Gram positive bacteria, including *S. aureus* and major CoNS from positive blood culture bottles.[32,33] In this assay, there are also targets for several common resistance genes found in Gram positive pathogens, including *mecA*. A recent study reported that, compared to conventional laboratory methods, the Verigene BC-GP assay demonstrated 100% sensitivity and specificity for the identification of *S. aureus* and *S. epidermidis,* and detection of the *mecA* gene.[34]

Compared to studies describing laboratory performance, there are fewer studies describing the clinical and economic impact of rapid molecular assays in patients with *S. aureus* bacteraemia. One prospective Australian study assessed the impact of using the Cepheid Xpert MRSA/SA BC test on antimicrobial prescribing in 151 patients with GPC detected in blood cultures.[35] These authors found that rapid detection of MRSA by the Cepheid Xpert MRSA/SA allowed appropriate institution of vancomycin therapy in 54% of patients, and cessation of inappropriate antibiotics in 16% of patients with bacteraemia due to CoNS.[35] In a similar study, Parta *et al*. also found that the use of the Cepheid Xpert MRSA/SA BC significantly reduced the use of antimicrobial therapy in patients with CoNS bacteraemia compared to patients who had their *S. aureus* bacteraemia diagnosed using traditional methods (76% versus 55%; $p < 0.01$).[27] In addition, a recent study by Frye *et al*. found that the time to identification of MSSA, MRSA and CoNS in positive blood cultures was significantly reduced using rapid molecular testing compared to conventional methods (47.3 hours pre-implementation versus 34.1 hours post-implementation; $p < 0.0001$), even when molecular testing was batched and performed once or twice daily.[36] Notably however, this study found that use of rapid testing had no impact on clinical outcomes, including time to optimal antimicrobial therapy, length of hospital stay or mortality.[36] These authors suggested that introduction of rapid molecular testing alone would not improve clinical outcomes unless other factors were incorporated, including 'on-demand' molecular testing, active clinician notification of test results, and the inclusion of rapid test results into an existing antimicrobial stewardship programme.[36]

Although molecular testing for the diagnosis of *S. aureus* bacteraemia potentially offers a faster turnaround time than conventional methods, there are a number of factors to consider prior to the routine implementation of such technology in

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