Journal of Antimicrobial Chemotherapy (2008) **61**, 1277–1280
doi:10.1093/jac/dkn122
Advance Access publication 20 March 2008

JAC

Microbiological evaluation of a new growth-based approach for rapid detection of methicillin-resistant *Staphylococcus aureus*

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Received 18 January 2008; returned 12 February 2008; revised 26 February 2008; accepted 26 February 2008

***Objectives*: Recently, a rapid screening tool for methicillin-resistant *Staphylococcus aureus* (MRSA) has been introduced that applies a novel detection technology allowing the rapid presence or absence of MRSA to be determined from an enrichment broth after only a few hours of incubation. To evaluate the reliability of this new assay to successfully detect MRSA strains of different origin and clonality, well-characterized *S. aureus* strains were tested in this study.**

***Methods*: More than 700 methicillin-susceptible and methicillin-resistant strains covering >90% of all registered European MRSA *spa* types within the SeqNet network were studied.**

***Results*: All 513 MRSA strains tested were recognized as methicillin-resistant: among these, 96 MRSA strains were from an institutional collection, each presenting a unique *spa* type. None of the 211 methicillin-susceptible strains were detected as positive.**

***Conclusions*: The new growth-based rapid MRSA assay was shown to detect without exception all MRSA strains of large collections of strains comprising highly diverse genetic backgrounds, indicating that such a phenotypic test might be potentially more likely to cope with new strains.**

Keywords: staphylococci, MRSA, infection control, *spa* typing

Introduction

The increasing numbers of multidrug-resistant Gram-positive pathogens have generated worldwide concern in the medical community. In particular, methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of disease and healthcare expenditures in almost every continent. The emergence and spread of MRSA has been shown to be associated with both hospital- and community-acquired infections. Effective treatment options for these infections are limited and the situation may become more severe soon. For these reasons, a proactive management of MRSA in healthcare facilities is needed.[1,2]

Active screening and compliance to appropriate infection control activities have been shown to play an important role in the control of MRSA.[1] Rapid diagnostic tests have the potential to make efforts even more effective. Thus, infection prevention has taken a step forward with the introduction of various tests for rapid identification of MRSA carriers.[1,2]

In 2006, a new rapid method based on a novel bioluminescence detection technology for the rapid detection of MRSA directly from specimens was published.[3] An improved version of this assay, the 3M™ BacLite™ Rapid MRSA Test (3M Company, Maplewood, MN, USA), was recently introduced, which allows the presence or absence of MRSA to be determined within 5 h. Although the clinical performance has been previously analysed,[4,5] so far no data are available on the detection of MRSA strains with highly diverse genetic backgrounds.

The aim of this study was to assess the reliability of this assay to successfully detect MRSA strains circulating currently in Germany and other parts of Europe on the basis of several well-characterized *S. aureus* strain collections. For this purpose, *S. aureus* strains of different origin and clonality comprising 724 methicillin-susceptible and methicillin-resistant strains comprising >90% of all registered European MRSA *spa* types within the SeqNet network (www.SeqNet.org) were tested in this study.

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Materials and methods

All staphylococcal strains were freshly isolated from clinical material at the University of Münster or during the course of various multicenter studies. One hundred and sixty-four strains isolated from patients with *S. aureus* bacteraemia (including 12 MRSA strains) as well as 50 isolates from the anterior nares of patients who did not subsequently develop *S. aureus* bacteraemia in a subsequent observation period (4 MRSA strains), were included into this study. Apart from 28 isolates from 14 patients (*S. aureus* first recovered from the anterior nares and subsequently from blood, one infected with MRSA), only one isolate per patient was tested. In addition, 96 MRSA strains, each presenting a unique *spa* type, were selected from our institutional collection (Table 1). Furthermore, four hundred MRSA isolates were collected during the course of a recent multicenter study, also including community-acquired MRSA. In total, the 724 *S. aureus* strains tested comprised 211 methicillin-susceptible *S. aureus* (MSSA) and 513 MRSA strains.

If the biochemical identification of staphylococcal isolates using the ATB 32 Staph gallery (bioMérieux, Marcy l'Étoile, France) was ambiguous or categorized as unacceptable, partial 16S rRNA gene and RNA polymerase B (*rpoB*) gene sequencing was performed as described previously.[6] Isolates were confirmed to be methicillin-resistant by detection of the *mecA* gene.

Table 1. Grouping of *spa* types tested in this study into *spa* clonal complexes (*spa*-CCs)

spa-CC[a]	*spa* types[b]	Putative MLST types[c]
001	t001, t002, t003, t010, t035, t039, t041, t045, t055, t057, t066, t105, t106, t109, t110, t143, t149, t151, t264, t265, t422, t820, t892, t1018	ST-5, ST-45, ST-46, ST-222, ST-225, ST-228, ST-231, ST-111, ST-228
004	t004, t028, t029, t033, t040, t043, t061, t065, t141, t142, t266, t911	ST-45, ST-45, ST-46
015	t015, t031, t069, t073, t102, t116, t133	ST-45
024	t008, t009, t024, t036, t051, t052, t068, t113, t115, t139, t146, t243, t305	ST-8, ST-235, ST-247, ST-250, ST-254, ST-247, ST-250
032	t005, t022, t032, t107, t290, t379, t432, t794	ST-22, ST-23, ST-60
037	t011, t030, t037, t047, t108, t135, t137, t138	ST-30, ST-239, ST-240, ST-241, ST-246
038	t038, t161, t247	ST-45
044	t042, t044, t131	ST-80
Singletons	t012, t091, t101, t104, t163, t321, t372, t417, t431, t907	ST-7, ST-30, ST-22

[a]Naming of *spa*-CC was derived from the group founder.
[b]t026, t103, t111, t132, t145, t282, t322 and t1007 were excluded from determination of the *spa*-CCs due to low number of repeats.
[c]Putative MLST types were taken from the public SeqNet.org *spa* server database.

To determine the clonal lineages of MRSA strains, the x region of the *spa* gene was amplified by PCR with primers 1095F (5′-AGACGATCCTTCGGTGAGC-3′) and 1517R (5′-GCTTTTG CAATGTCATTTACTG-3′). DNA sequences were obtained with an ABI 377 sequencer (Applied Biosystems, Foster City, CA, USA). *spa* types were determined using the Ridom StaphType software version 1.3 (Ridom GmbH, Würzburg, Germany), and *spa* clonal complexes (*spa*-CCs) were assigned by using the BURP algorithm.[7]

The 3M™ BacLite™ Rapid MRSA Test is a novel culture-based test for the detection of MRSA performed on a semi-automated system comprising a sample processor and a luminometer. The assay consists of three successive selectivity steps: selective enrichment in a proprietary broth containing cefoxitin (2 mg/L) and colistin (50 mg/L), immuno-magnetic extraction using a highly specific anti-*S. aureus* monoclonal antibody and selective lysis using lysostaphin. The selectivity steps are followed by a detection step using a highly sensitive cell marker, adenylate kinase (AK). AK is an essential enzyme found in all living cells, which regulates energy provision by catalysing the equilibrium reaction ATP + AMP = 2 ADP. By supplying a continual source of purified ADP, this assay drives the AK reaction to generate up to 40 000 ATP molecules per min. These amplified levels of ATP are measured using the luminometer supplied with the system.

For the present study, test isolates were streaked on sheep blood agar plates and grown at 37°C to confirm purity. For each isolate, one to three colonies were picked and suspended in sterile saline (0.9%) to achieve a turbidity equivalent to that of a 0.5 McFarland standard. Usually, 43 isolates were processed in each run. The 3M™ BacLite™ Rapid MRSA Test was performed according to the instructions of the manufacturer. In brief, 10 μL of each prepared bacterial suspension was transferred into a vial containing 1 mL of selective enrichment broth. Vials were incubated at 37°C for 2 h before 150 μL aliquots of each test sample were transferred into two adjacent wells of a 96-well assay plate, each containing 20 μL of capture reagent. MRSA cells bound to the monoclonal antibody on the capture reagent were then extracted from the sample matrix using the sample processor and concentrated in 100 μL of broth. One well for each sample was processed automatically for a baseline signal (T0) in the BacLite luminometer. After a further incubation period of 2 h at 37°C, the second well for each sample was processed in the same way (T2 reading). Results are expressed in relative light units and interpreted as either a positive or negative screen result by a software embedded algorithm. Positive and negative controls as well as reagent and broth controls were included in each test run. A run took ~5 h with a total hands-on time of 45–50 min (<1.5 min per tested isolate).

Results and discussion

Analysing a total of 724 *S. aureus* strains, all 513 MRSA strains tested were recognized as MRSA, whereas none of the 211 MSSA strains was detected positive by the 3M™ BacLite™ Rapid MRSA Test. These results are particularly impressive as the institutional MRSA strain collection used in this study represents more than 90 different *spa* types covering >90% of all registered European MRSA *spa* types within the SeqNet network (Table 1). Beside several singletons, the MRSA enrolled in this study were grouped into eight *spa* clonal complexes (Table 1). Thus, a very large number of MRSA strains with different genetic backgrounds were recognized as MRSA using this novel method.

Hospitals and other healthcare facilities across the world are faced with alarming rates of infections caused by MRSA. Continuous spread of these pathogens requires efficient strategies for infection control. Early identification of MRSA carriers among hospitalized patients is crucial to prevent its spread.[1] Therefore, rapid availability of laboratory results is of utmost importance. However, conventional screening methods require prolonged incubation and confirmatory testing. During this time, MRSA-negative patients may be held in unnecessary isolation, whereas unidentified MRSA-positive individuals remain a hidden reservoir for cross-infection. A rapid negative result should allow more effective use of hospital isolation resources, whereas a rapid positive result should help reduce the spread of the infection and MRSA infection rates.[2]

In the past few years, several in-house and commercial rapid MRSA assays based on molecular techniques have been introduced for the detection of this pathogen directly from the specimen, mostly from the anterior nares. The first molecular assays developed were based on the detection of an *S. aureus*-specific sequence and the *mecA* gene, which encodes methicillin resistance.[8] These tests are difficult to use for the direct detection of MRSA from non-sterile specimens, such as nasal samples, because of the likely co-presence of MSSA and methicillin-resistant coagulase-negative staphylococci.[9] In a setting of low prevalence of MRSA, a molecular test targeting the *mecA* and an *S. aureus*-specific gene in parallel applied directly to clinical specimens would result in a high number of false positives and unacceptable performance.[9]

This technical limitation has been overcome in some assays, by linking detection of the presence of the *mecA* gene with detection of the neighbouring chromosome-borne *orfX* gene.[10,11] In that approach, regions near the integration site of SCC*mec* were targeted as surrogate markers instead of the *mecA* gene itself. However, these flanking regions are known to be more heterogeneous than assumed so far.[10] Thus, false-negative results due to variations within the primer binding sites may occur. Moreover, false-positive results due to the detection of DNA from non-viable MRSA, deletions or replacement of the *mec* region *in vivo* or ‘ghost sequences’ such as partial SCC*mec* sequences in MSSA can occur, albeit their incidence in the routine clinical setting is as yet unclear.[12] Despite the technical improvements in recent molecular-based assays, their high costs and relatively high operator skill requirement remain obstacles to their widespread routine use.

The ability of a test to detect a broad range of MRSA clones is particularly important for an assay that may be used across a wide geographic region as tests with gaps in detection could potentially ‘select out’ strains whose spread would be uncontrolled.

Beside a variety of method-inherent limitations, rapid DNA-based methods amplify the nucleic acid and not the organism, which means the MRSA strain is unavailable for further characterization, such as determination of the resistance profile and strain typing. In contrast, applying the rapid growth-based assay tested here, any further examinations to characterize the respective strains will be possible from the enrichment broth.

For the 3M™ BacLite™ Rapid MRSA Test, a diagnostic sensitivity of 94.6% and diagnostic specificity of 96.9% for nasal screening swabs and 95.9% sensitivity and 88.8% specificity for groin screening swabs, respectively, have been reported.[4,5] The analytical limit of detection of the assay was shown to be ≤ 94 cfu (3M™ BacLite™ Rapid MRSA Test, Instructions for Use).[4,5]

Here, the new growth-based rapid MRSA assay was shown to detect without exception all MRSA strains of large collections of strains comprising highly diverse genetic backgrounds. Such a phenotypic test might be potentially more likely to cope with new strains. Further studies are warranted to evaluate this method using clinical specimens.

Acknowledgements

We would like to thank A. Eggemann for excellent experimental assistance.

Funding

This work was supported in part by a grant from the Bundesministerium für Bildung und Forschung (BMBF), Germany (Pathogenomic Plus Network PTJ-BIO/0313801B). The device and the respective assay consumables were given to us by 3M for this study.

Transparency declarations

Two of the authors (A. W. F. and K. B.) delivered independent scientific presentations at 3M-supported symposia. C. v. E. is a member of 3M’s Scientific Advisory Board. All other authors: none to declare.

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