AKADEMISKA SJUKHUSET

Evaluation of the eazyplex® MRSAplus assay for rapid identification of methicillin resistant Staphylococcus aureus (MRSA) and detection of the virulence factor Panton-Valentine leukocidin (PVL) in clinical isolates.

Anna Hill[1], Anna-Karin Smekal[1], Hilde Merete Riedel[1,2]

[1]*Uppsala University Hospital, Department of Clinical Microbiology, Uppsala, Sweden.*
[2]*Uppsala University, Department of Medical Sciences, Section of Clinical Bacteriology, Uppsala, Sweden.*

INTRODUCTION

The spread of methicillin resistant Staphylococcus aureus (MRSA) is a major and growing problem in healthcare settings. Rapid diagnostic methods are of great importance to fight nosocomial transmission between patients and to optimize treatment for individual patients with invasive MRSA infections. Patients with invasive MRSA infections have significantly higher mortality and morbidity rates compared to patients with non-MRSA invasive infections (1, 2).

Panton-Valentine leukocidin (PVL) is an exotoxin produced by some *S aureus* isolates, mediating death of neutrophil granulocytes and monocytes hence leading to leukopenia (3). Regardless of their resistance phenotype, PVL positive S aureus are commonly associated with chronic/recurrent skin and soft tissue infections as well as rare but severe necrotizing pneumonia (4), often in previously healthy young people. In these cases altered antibiotic treatment is required why rapid detection of the PVL-gene can improve clinical outcome.

The aim of this study was to test a fast detection assay, eazyplex® MRSAplus, for identification of the species *S aureus*, the resistance genes *mecA* and *mecC*, and the *lukS-lukF* gene (PVL) in clinical isolates.

MATERIALS AND METHODS

The eazyplex® MRSAplus assay (Amplex Diagnostics) is a rapid confirmatory test for the specific detection of MRSA and PVL from bacterial colonies, based on loop-mediated isothermal amplification (LAMP) and real-time detection of the corresponding genes or species (the species *Staphylococcus aureus*, the resistance genes *mecA* and *mecC*, the *lukS-lukF* gene). A collection of 73 Staphylococcus isolates previously confirmed by molecular methods (in-house PCR targeting *nuc*, *Sa442* and *mecA* genes) were used in the evaluation.

Of the 73 isolates tested (Table 1), 7 were reference strains, 5 from Culture Collection, University of Gothenburg (CCUG) and 2 from American Type Culture Collection (ATCC). 6 isolates were coagulase negative Staphylococcal species, 13 were known MRSA isolates from our culture collection and 18 were consecutive collected isolates. 5 isolates were consecutive MSSA-isolates. 18 were PVL-positive isolates, of which 9 had known PVL and 9 were found PVL-positive during this study, all 18 verified with in house PCR (5,6). 11 were mecC-positive isolates.

The assay was performed according to the manufacturer's instructions on Genie II (Amplex Diagnostics). A small part of a single bacterial colony is suspended in buffer, heated and transferred to a test strip which is placed into Genie®II (Fig 1 and 2). In case of match, the target gene is amplified which will cause a fluorescent signal registered as a positive test result (more than 5000 units). The whole procedure takes about 25 minutes from start to result.

Figure 1. A small part of a single bacterial colony is suspended in 500 µl RALF buffer and incubated at 99ºC for 2 min. 25 µl of the RALF suspension is transferred to each single tube of the test strip.

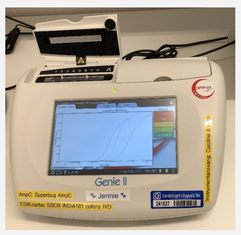

Figure 2. LAMP reaction and detection is monitored in real time by the GENIE®II instrument.

Table 1: Reference strains and isolates used in this study

67	**Staphylococcus aureus isolates as presented below**
7	*Reference strains (CCUG 67181, 35601, 15915, 47167, 17621 and ATCC 13566, 19095)*
13	*mecA-MRSA (previously known isolates)*
11	*mecC-MRSA (previously known isolates)*
18	*PVL-positive isolates*
23	*Consecutive clinical isolates*
6	**Coagulase negative Staphylococcus species isolates**

Isolates were collected from our own culture collection, from Karolinska University Hospital (Solna), Skåne University Hospital (Lund) and from Public Health Agency of Sweden.

RESULTS

The assay identified all target genes correctly.

Thus, a sensitivity of 100% for the target genes *mecA*, *mecC* and *lukS-lukF* (PVL) were obtained.

No false positive results were observed resulting in a specificity of 100% for *mecA*, *mecC* and *lukS-lukF* (PVL).

Table 2: Performance data

Staphylococcus aureus	**SA+**	**SA-**	**Σ**	
eazyplex® +	67	0	67	
eazyplex® -	0	6	6	**Sensitivity 100%**
Σ	67	6	73	**Specificity 100%**

mecA	***mecA+***	***mecA-***	**Σ**	
eazyplex® +	37	0	37	
eazyplex® -	0	36	36	**Sensitivity 100%**
Σ	37	36	73	**Specificity 100%**

mecC	***mecC+***	***mecC-***	**Σ**	
eazyplex® +	11	0	11	
eazyplex® -	0	62	62	**Sensitivity 100%**
Σ	11	62	73	**Specificity 100%**

PVL(lukS-lukF)	**PVL+**	**PVL-**	**Σ**	
eazyplex® +	18	0	18	
eazyplex® -	0	3	3	**Sensitivity 100%**
Σ	18	3	21	**Specificity 100%**

CONCLUSIONS

The eazyplex® MRSAplus assay (Amplex Diagnostics) obtained 100% sensitivity and specificity for the target genes *mecA*, *mecC* and *lukS-lukF* (PVL) for the Staphylococcus isolates.

In conclusion, the eazyplex® LAMP technique is a rapid (only 20-30 minutes) and reliable method demonstrating high sensitivity and specificity for the identification of species, resistance and toxin genes in clinical isolates. The method is easy to use and timesaving for the laboratory. The rapid results provided can help optimizing patient treatment.

REFERENCES

1. Kim SH *et al* (2003). *Outcome of Staphylococcus aureus bacteremia in patients with eradicable foci versus noneradicable foci*. Clin Infect Dis 37(6):794–799.
2. Lodise TP, McKinnon PS (2005). *Clinical and economic impact of methicillin resistance in patients with Staphylococcus aureus bacteremia*. Diagn Microbiol Infect Dis 52(2):113–122.
3. Shallcross LJ, Fragaszy E, Johnsson AM and Hayward AC (2013). *The role of Panton-Valentine leucocidin toxin in staphylococcal disease: a systematic review and meta-analysis*. Lancet Infect Dis 13:43-54.
4. Saeed K *et al* (2018). Hot topics in necrotising skin and soft tissue infections. Int J Antimicrob Agents 52(1):1-10.
5. Fang H and Hedin G (2003). *Rapid screening and identification of methicillinresistant Staphylococcus aureus from clinical samples by selective-broth and real-time PCR assay*. J Clin Microbiol 41(7):2894-99.
6. Nilsson P, Alexandersson H and Ripa T (2005). Use of broth enrichement and real-time PCR to exclude the presence of methicillin-resistant Staphylococcus aureus in clinical samples: a sensitive screening approach. Clin Microbiol Infect 11(12): 1027-34.

Contact information: hilde.riedel@akademiska.se

UPPSALA UNIVERSITY HOSPITAL