JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Letter to the Editor

Rapid detection of methicillin-resistant *Staphylococcus aureus* and SCC*mec* types from nasal swabs by multiplex PCR

Fernanda Sampaio Cavalcante[1], Eliane de Dios Abad[2], Ana Carolina Fonseca Guimarães[1], Simone Saintive Barbosa[2], Marcia Ribeiro[2], Dennis de Carvalho Ferreira[3], Kátia Regina Netto dos Santos[1]

[1] *Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil*
[2] *Instituto de Pediatria e Puericultura Martagão Gesteira, Universidade Federal do Rio de Janeiro; Rio de Janeiro, Brazil*
[3] *Programa de Pós-Graduação em Odontologia, Faculdade de Odontologia, Universidade Veiga de Almeida, Rio de Janeiro, Brazil*

Key words: SCC*mec* types; MRSA; oxacillin selective broth; nasal swab.

J Infect Dev Ctries 2016; 10(2):195-198. doi:10.3855/jidc.7414

(Received 12 July 2015 – Accepted 28 August 2015)

Dear Editor,

Staphylococcus aureus is the most important cause of infection worldwide and the majority of such infections are related to methicillin-resistant *S. aureus* (MRSA) [1]. MRSA isolates present a staphylococcal cassette chromosome *mec* (SCC*mec*), and types II, III and IV are the most frequent [2,3,4]. Epidemiological studies conducted in recent years have shown that SCC*mec* IV isolates, mainly related to USA300 and European clones, are found in about 80% of MRSA isolates collected from community and hospital settings in Europe [2]. In the USA, MRSA lineages carrying SCC*mec* II are also very frequent [3], while the SCC*mec* III isolates are widespread in many countries and are found in hospitals on all continents [4].

MRSA colonization is a worldwide health problem. Moreover, in 2015 the World Health Organization prepared, a global action plan against antimicrobial resistance which involves a series of strategies, including a significant innovation strategy for the diagnosis and control of pathogens [5]. A rapid identification of MRSA isolates and their SCCmec types could help track MRSA clones, allowing better control of infections as well as improvements in patient management. Here, we propose a method to detect MRSA and distinguish the types of SCC*mec* from nasal swabs after culture in pre-enriched selective broth.

The study

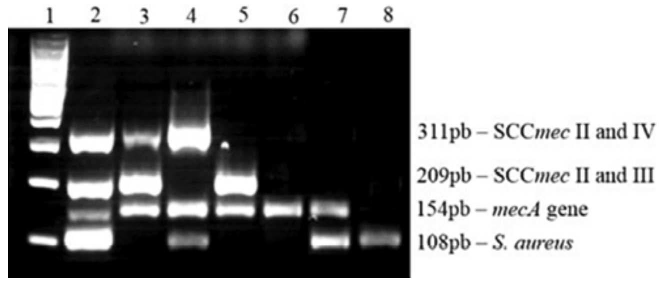
As part of a larger study, 362 nasal swabs were collected from February 2012 to November 2013, in an outpatient referral centre for atopic dermatitis in Rio de Janeiro. Swabs were collected from 159 pediatric outpatients and 203 relatives and then placed on mannitol-salt agar (MSA) (Oxoid Ltd, Basingstoke, England). After which, the swabs were inoculated into 5ml of selective broth (SB) (Mueller-Hinton broth Difco, Detroit, USA) containing 7% [wt/vol] NaCl and 2µg/ml of oxacillin [Sigma-Aldrich, St. Louis, USA] and incubated for 24h at 35° C [6]. MSA plates were incubated for 48h at 35° C. Both bacterial growths were transferred to blood agar and identified by standard tests. Methicillin-resistance was accessed by the cefoxitin disk test (Oxoid Ltd, Basingstoke, England) [7].

DNA was extracted by boiling and SCC*mec* typing was performed [8]. Nontypeable isolates were submitted to DNA extraction with guanidium thyocyanate and SCC*mec* typing [9]. Based on a previous study conducted by our group which showed that both SB and MSA presented specificity above 99% for MRSA detection [6], all isolates identified as MRSA by either method were considered true positive (gold standard detection). Multiplex PCR was carried out using DNA from SB liberated by boiling [6] plus 100µM of dNTPs (Life Technologies), *Taq* DNA polymerase (1.5U), Tris-HCl (20mM), KCl (50mM), $MgCl_2$ (2mM) (Biotools, Madrid, Spain) and the primers: Sa1/Sa2 (0.4µM) (*S. aureus* species) [10]; MRS1/MRS2 (1µM) [11], (*mec*A gene); MECIP2/MECIP3 (0.8µM) (SCC*mec* II and III) and CcrB1/CcrB2 (0.8µM) (SCC*mec*II and IV) [8]. Amplification conditions included 4min/94 ºC, 30 cycles of 94 ºC/30s, 53 ºC/30s and 72 ºC/1min and a final extension of 72º/4min.

The SB-MPCR method was validated using previously published control strains. These strains had been grown in SB until reaching turbidity 0.5 McFarland. DNA extraction and PCR procedures were as described above. Additionally, 35 MRSA clinical isolates previously characterized in relation to minimum inhibitory concentrations (MIC) to oxacillin and SCC*mec* types were submitted to SB-MPCR for simulation. The oxacillin MICs for the MRSA isolates recovered in the present study were also assessed [7].

The SB-MPCR method detected 42 MRSA isolates. Among the swabs that were MRSA negative according to this method, 180 coagulase-negative *Staphylococcus* (CoNS), 54 Gram negative bacteria (GNB) and nine methicillin-susceptible *S. aureus* (MSSA) isolates were detected; however 77 did not grow in the SB. On the other hand, routine tests detected 44 MRSA, 173 CoNS, 132 MSSA and six GNB. Considering that the isolates recovered from both SB and routine tests were true positives, 51 MRSA isolates were detected in this study (Table 1).

Figure 1. Gel electrophoresis of *Staphylococcus* spp. isolates submitted to multiplex PCR from selective broth (MPCR-SB).



1 - molecular size marker (100 bp ladder); 2 - *S. aureus* SCC*mec* II; 3, 5 and 6 – methicillin-resistant coagulase-negative *Staphylococcus*; 4 - *S. aureus* SCC*mec* IV; 7 - *S. aureus* SCC*mec* nontypeable; 8 - methicillin-susceptible *S. aureus*.

Nine MRSA isolates that were not-recovered in SB presented oxacillin MICs<4µg/ml (ranging from 0.5 to 1 µg/ml) (Table 2). Similar results were obtained for the clinical MRSA isolates (Table 2). The SB-MPCR method identified the SCC*mec* type of 35 MRSA isolates among a total of 42 isolates detected (Table 1). Three isolates carried one of the type II, III or nontypeable cassette each, while the other 32 carried the SCC*mec* IV (Figure 1). Seven isolates carrying the

Table 1. Results obtained from culture in selective broth followed by the multiplex PCR (SB-MPCR) method in comparison to the routine tests for the detection of methicillin-resistant *Staphylococcus aureus* and their SCC*mec* types from 362 nasal swabs.

Detection method	MRSA isolates (total=51)						MRSA isolates carrying SCC*mec* IV (total=48)						MRSA isolates carrying other SCC*mec* types[h] (total=3)					
	TP[a]	FN[b]	SE[c]	SP[d]	PPV[e]	NPV[f]	TP	FN	SE	SP	PPV	NPV	TP	FN	SE	SP	PPV	NPV
Routine tests	44	7	86.2%	100%	100%	97.7%	40	8	83.3%	100%	100%	97.4%	3	0	100%	100%	100%	100%
SB-MPCR	42	9[g]	82.3%	100%	100%	97.1%	32	16[i]	66.6%	100%	100%	94.9%	3	0	100%	100%	100%	100%

[a] True positive; [b] False negative; [c] Sensibility; [d] Specificity; [e] Positive predictive value; [f] Negative predictive value; [g] All these nine MRSA isolates presented MIC to oxacillin <4ug/ml; [h] Isolates presented SCC*mec*types II and III and a nontypeable cassette; [i]ncluding nine isolates that did not grow in selective broth and 7 isolates that were misidentified.

Table 2. Comparative results obtained from culture in selective broth followed by the multiplex PCR (SB-MPCR) method in clinical MRSA isolates carrying different SCC*mec* types and presenting different oxacillin MIC values in the MRSA isolates detected in the present study.

SCC*mec* type/ oxacillin MIC (nº of MRSA isolates)	MRSA isolates				SCC*mec* types detected			
	TP[a]	FN[b]	SE[c]	SP[d]	TP	FN	SE	SP
Clinical isolates (total=35)								
II/ ≥4µg/ml (5)	5	0	100%	100%	5	0	100%	100%
III/≥4µg/ml (10)	10	0	100%	100%	10	0	100%	100%
IV/ <4µg/ml (10)	7	3[e]	70%	100%	6	4	60%	100%
IV/ ≥4µg/ml (10)	10	0	100%	100%	9	1	90%	100%
Total	32	3	91.4%	100%	30	5	85.7%	100%
Isolates detected in the present study (total=51)								
IV/ ≥4µg/ml (39)	39	0	100%	100%	32	7	82%	100%
IV/ <4µg/ml (9)	0	9[f]	na	na	0	9	na	na
Other/ ≥4µg/ml (3)	3	0	100%	100%	3	0	100%	100%

[a]True positive; [b]False negative; [c]Sensibility; [d]Specificity; [e]These isolates did not grow in selective broth and presented oxacillin MICs ranging from 0.5 to 1µg/ml; [f] These isolates did not grow in selective broth and presented oxacillin MICs ranging from 0.5 to 2µg/mL; na - not applicable

SCC*mec* IV showed only the *mec*A gene band in the test.

The detection of MRSA from clinical specimens has been proposed elsewhere [6,12,13]. However, these proposed methods were not able to distinguish the different SCC*mec* types. Here, we successfully described a rapid PCR-based method to detect the *mec*A gene and segments of the SCC*mec* types II, III and IV in MRSA isolates grown in a selective broth.

In the present study conventional and SB-MPCR methods presented excellent specificity although failed to detect some MRSA isolates. The SB-MPCR presented a sensitivity of 82.3% to detect MRSA. This value was similar to the one obtained by Yam *et al*. [13] in the evaluation of a commercial PCR-based method for MRSA detection (83.3%). Our method failed to detect nine isolates, whose oxacillin MICs were lower than 4µg/ml (range of 0.5 to 1µg/ml), values commonly found among community isolates [14]. However, MRSA clinical isolates with very low oxacillin MICs are not common. Moreover, to investigate the role of MIC in the accuracy of the SB-MPCR method, 35 MRSA clinical isolates previously characterized were analyzed by this method and similar results were found, confirming that type IV isolates presenting very low oxacillin MICs cannot be detect by the SB-MPCR method. According to Andriesse *et al*. [12], false negative in conventional methods are related to low counts in community patients. They found 86% of sensitivity for bacterial culture agreeing with the present study.

The SB-MPCR method was also designed to distinguish the types of SCC*mec* prevalent worldwide, characterizing the methicillin resistance quickly. SB-MPCR identified the SCC*mec* types in 68.8% of the 51 MRSA isolates. Seven SCC*mec* IV isolates showed only the *mecA* gene band. The great genetic diversity of SCC*mec* IV, probably due to recombination and enhanced mobility, is a possible explanation for the difficulty to detect these isolates [15].

To avoid false-positive results from the co-existence between methicillin-resistant CoNS and MSSA isolates, Schuenck *et al*. [6] recommended 4ug/ml of oxacillin to inhibit MSSA. However, in the present study, this concentration inhibited type IV isolates (data not shown). Although dubious results can occur, Schuenck and coworkers did not find any questionable results even at concentrations of 2ug/ml of oxacillin.

A number of studies using various multiplex PCRs for SCC*mec* typing have been described [8,9]. These techniques present good sensitivity and specificity and can identify a large number of SCC*mec* types. However, these methods were designed to detect only SCC*mec* types after the identification of *S. aureus* followed by DNA extraction, which requires a minimum of 72 hours.

Here we present a rapid detection of MRSA and SCC*mec* types from nasal swabs which is faster than the conventional methodologies. The SB-MPCR showed high specificity, low cost and a sensitivity similar to the commercial PCR-based methods. Detection of *S. aureus* species, methicillin resistance and SCC*mec* type was performed within 24h after culture in selective broth. False negative results were associated to isolates with very low oxacillin MICs, situations that are not clinically common. Moreover, the method characterized the SCC*mec* types of the majority of isolates, showing that it can provide fast and reliable results to help control MRSA infections.

Acknowledgements
This study was supported by Brazilian grants from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES), Fundação Universitária José Bonifácio (FUJB) and Programa de Núcleos de Excelência (PRONEX).

References

1. David MZ, Daum RS (2010) Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 23: 616-687.
2. Rolo J, Miragaia M, Turlej-Rogacka A, Empel J, Bouchami O, Faria NA, Tavares A, Hryniewicz W, Fluit AC, de Lencastre H; CONCORD Working Group (2012) High genetic diversity among community-associated *Staphylococcus aureus* in Europe: results from a multicenter study. PLoS One 7: e34768.
3. Haque NZ, Arshad S, Peyrani P, Ford KD, Perri MB, Jacobsen G, Reyes K, Scerpella EG, Ramirez JA, Zervos MJ (2012) Analysis of pathogen and host factors related to clinical outcomes in patients with hospital-acquired pneumonia due to methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 50: 1640-1644.
4. Baines SL, Holt KE, Schultz MB, Seemann T, Howden BO, Jensen SO, van Hal SJ, Coombs GW, Firth N, Powell DR, Stinear TP, Howden BP (2015) Convergent adaptation in the dominant global hospital clone ST239 of methicillin-resistant *Staphylococcus aureus*. MBio 6: e00080.
5. Hoffman SJ, Outterson K, Røttingen JA, Cars O, Clift C, Rizvi Z, Rotberg F, Tomson G, Zorzet A (2015) An international legal framework to address antimicrobial resistance. Bull World Health Organ 93: 66.
6. Schuenck RP, Lourenço MC, Iório NL, Ferreira AL, Nouér SA, Santos KR (2006) Improved and rapid detection of methicillin-resistant *Staphylococcus aureus* nasal carriage

using selective broth and multiplex PCR. Res Microbiol 157: 971-975.
7. Clinical Laboratory Standards Institute (CLSI) (2010) Performance standards for antimicrobial susceptibility testing; Twentieth informational supplement. CLSI document M100eMS20. Wayne [PA]: Clinical Laboratory Standards Institute.
8. Milheiriço C, Oliveira DC, de Lencastre H (2007) Multiplex PCR strategy for subtyping the staphylococcal cassette chromosome *mec* type IV in methicillin-resistant *Staphylococcus aureus*: 'SCC*mec* IV multiplex'. J Antimicrob Chemother 60: 42-48.
9. Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, Hiramatsu K (2007) Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. Antimicrob Agents Chemother 51: 264-274.
10. Martineau F, Picard FJ, Roy PH, Ouellette M, Bergeron MG (1998) Species-specific and ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus*. J Clin Microbiol 36: 618-623.
11. Del Vecchio VG, Petroziello JM, Gress MJ, McCleskey FK, Melcher GP, Crouch HK, Lupski JR (1995) Molecular genotyping of methicillin-resistant *Staphylococcus aureus* via fluorophore-enhanced repetitive-sequence PCR. J Clin Microbiol 33: 2141-2144.
12. Andriesse GI, van Rijen M, Bogaers D, Bergmans AM, Kluytmans JA (2009) Comparison of two PCR-based methods and conventional culture for the detection of nasal carriage of *Staphylococcus aureus* in pre-operative patients. Eur J Clin Microbiol Infect Dis 28: 1223-1226.
13. Yam WC, Siu GK, Ho PL, Ng TK, Que TL, Yip KT, Fok CP, Chen JH, Cheng VC, Yuen KY (2013) Evaluation of the Light Cycler methicillin-resistant *Staphylococcus aureus* (MRSA) advanced test for detection of MRSA nasal colonization. J Clin Microbiol 51: 2869-2874.
14. Matos PD, Schuenck RP, Cavalcante FS, Caboclo RM, Santos KR (2010) Accuracy of phenotypic methicillin susceptibility methods in the detection of *Staphylococcus aureus* isolates carrying different SCC*mec* types. Mem Inst Oswaldo Cruz 105: 931-934.
15. Hill-Cawthorne GA, Hudson LO, El Ghany MF, Piepenburg O, Nair M, Dodgson A, Forrest MS, Clark TG, Pain A (2014) Recombinations in staphylococcal cassette chromosome *mec* elements compromise the molecular detection of methicillin resistance in *Staphylococcus aureus*. PLoS One 9: e101419.

Corresponding author

Kátia Regina Netto dos Santos
Laboratório de Infecção Hospitalar, Departamento de Microbiologia Médica
Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, CCS, Bloco I, Sala 010. Rio de Janeiro, RJ, Brazil. CEP: 21941-590
Phone: 55-21-2560-8344
Email: santoskrn@micro.ufrj.br

Conflict of interests: No conflict of interests is declared.